MICROBIOLOGICAL REVIEWS, Sept. 1985, p. 270–297
0146-0749/85/030270-28$02.00/0

Vol. 49, No. 3

Kinetics of Nutrient-Limited Transport and Microbial Growth

D. K. BUTTON

Institute of Marine Science, University of Alaska, Fairbanks, Alaska 99775

INTRODUCTION 270
GROWTH RATE 270
SUBSTRATE UPTAKE 272
Transport Mechanisms 273
Rate Constants 273
Kinetics Based on Continuous Culture 274
Rate, Biomass, and Yield 274
Saturation 275
Kinetics of Growth from Nutrient Uptake 275
KINETIC CONSTANTS 275
V_{max} and K_t 275
μ_{max} and K_μ 276
K_t and K_μ 276
Affinity 276
NON-GROWTH PHENOMENA 278
Endogenous Metabolism 278
Respiratory Loss 279
Substrate Thresholds 279
CELL QUOTA 280
EFFECTIVE DATA COLLECTION 280
Conditioning of Cells 280
Effective Concentrations 289
Reasonable Kinetic Constants 291
KINETIC DATA 291
Organization of Tabulations for Bioelements 291
ACKNOWLEDGMENTS 292
LITERATURE CITED 292

INTRODUCTION

Nutrient-limited growth is the usual state for most microorganisms of the environment. In aquatic systems, nutrients are usually absorbed until concentrations decrease to levels that are sufficient for the growth of only those organisms with good transport systems, or ones that can grow very slowly. Many terrestrial systems are also restricted by nutrient concentrations, because much organic carbon exists in recalcitrant macromolecules which are hydrolyzed slowly with extracellular enzymes. In nutrient-limited systems the rate of microbial growth is set either by ability to transport the nutrient of greatest need or by the rate of use of a previously stored reserve.

Kinetics is the science that relates microbial growth rates to the nutrient concentations on which they depend. It can be divided into two processes, the kinetics of concentration-limited accumulation of nutrients and the efficiency that these nutrients provide for growth. Nutrient uptake is often observed by the rate of nutrient transfer into organisms over time. Growth is observed in closed systems by the rate of proliferation of a population, or, in open systems, by the rate at which a population can be removed without disturbing the steady state or by injected tracer movement through it. Theoretical treatments provide the ties between nutrient transport concentrations, which limit the rate of transporter activity in given systems, and the rate of nutrient accumulation expressed as growth.

Reviews have appeared on membrane transport (3, 76, 130, 151), nutrient transport (17, 179, 192; Goldman and Glibert, *in Nitrogen in the Marine Environment*, in press), growth kinetics (13, 42, 43, 49, 54, 61, 67, 115, 148, 165), culture techniques (27, 215), and kinetic formulations (200). The purpose of this review is to bring theory together with experimental data that relate nutrient concentrations, nutrient fluxes, and microbial growth rates. Formulations are presented with common nomenclature throughout, and kinetic observations are organized in such a way that all reported observations regarding the accumulation ability of various organisms for a particular nutrient can be compared. In addition to comparing the kinetics of transport and growth of different microorganisms, the compilations are a test of the ability of the experimental systems to resolve the kinetics involved.

GROWTH RATE

The rate of growth expresses the change in population with time. This change depends on the population as well as time, so that

$$\frac{dX}{dt} = \mu X \quad (1)$$

where μ is the instantaneous growth rate constant. (Definitions and units are given in Table 1.) Integrating between initial and final populations

TABLE 1. Nomenclature

Symbol	Definition	Units
A	Substrate A	Mass
A^e	Effective concentration of A	Mass/liter
A^*	Concentration of radiolabeled portion of A at prevailing specific activity	Mass/liter
A_b	Concentration of A supplied as background	Mass/liter
A_0	Concentration of A in fresh medium	Mass/liter
A_{out}	Concentration of A outside cells	Mass/liter
$A^*_{out\ 0}$	Concentration of radioactive A outside cells at time zero	Mass/liter
A_{pool}	Concentration of soluble A inside organisms	Mass/mass
A_t	Threshold concentration of A for growth	Mass/liter
A'	Area	Square centimeters
A	Arrhenius constant	
$a_A{}^0$	Specific affinity or organisms for substrate A at subsaturating substrate concentration	Liters per gram of cells per hour
$a_A{}^{K\mu}$	Specific affinity at the Michaelis concentration	Liters per gram of cells per hour
$a_{h\nu}$	Specific affinity for light	Grams of $[CH_2O]$ per square centimeter per gram of cells per microeinstein
B	Substrate B (see A)	
D	Dilution rate, F/V	Per hour
D	Diffusivity	Square centimeter per second
E	Free enzyme	Mass per liter
E_t	Total enzyme	Mass per liter
E_a	Activation energy	Calories per mole
F	Flow rate	Liters per hour
I	Intensity of light	Microeinsteins per square centimeter per hour
j	Rate of solute movement across interface	Mass per gram of cells per hour
K_d	Disassociation constant	
K_μ	Michaelis or substrate concentration at half-maximal growth rate	Mass per liter
K_t	Michaelis or substrate concentration at half-maximal transport rate	Mass per liter
k	General rate constant; endogenous metabolism, respiration, and reaction rate	Variable
k_Q	Lower limit cell quota Q' at $\mu = 0$	Grams of substrate per gram of cells
k_{net}	Net rate constant, forward less reverse	
ν	Frequency	Per second
P	Product of substrate A	Mass
P'	Photosynthetic rate	Grams of carbon per gram of chlorophyll per second
Q	Product of substrate B	Mass
Q'	Cell quota	Grams of substrate per gram of cells
R	Ratio of radioactive to total substrate at specific activity supplied	
R	Gas constant	Calories per degree per mole
R_{EM}	Ratio of specified enzyme to cell membrane	Mass per mass
R_{MX}	Ratio of cell membrane to cell mass	Mass per mass
R_{TX}	Ratio of transport material to cell mass	Mass per mass
T	Temperature, transporter, or permease	Degrees centigrade and mass per liter
T_t	Total transporter, $T + TA$	Mass per liter
t	Time	
t_d	Doubling time	Per hour
τ	Birth time	Hours
$t_{1/2}$	Half-time for nutrient depletion	Hours
μ	Specific rate of growth	Per hour
μ_{max}	Maximal growth rate	Per hour
v	Rate of product formation from substrate	Mass per liter per hour
v_A	Specific substrate uptake rate	Mass substrate accumulated per mass substrate in cells, per hour
v_{net}	Total rate of substrate uptake less leakage	Mass A per liter per hour
v^S	Specific substrate uptake rate	Grams of substrate taken up per gram of cells per hour
V	Volume	Liters
V_{max}	Maximal rate of uptake	Mass substrate per liter per hour
$V^A{}_{max}$	Maximal specific rate	Mass substrate per mass substrate in cells per hour
$V^S{}_{max}$	Maximal specific uptake rate	Grams of substrate per gram of cells per hour
V'_{max}	A constant related to V_{max}	
X	Biomass expressed in wet weight	Mass per liter
X_N	Biomass	Organisms per liter
ξ	Partition coefficient mass ratio in phases; also, absorbed irradiance	
Y_f	Fluid portion of cells	Mass per mass
Y_g	Yield for growth, i.e., cells produced from substrate used for growth processes	Mass per mass
Y_S	Solid portion of cells	Mass per mass
Y_{XA}	Yield of cells produced per substrate consumed	Mass per mass
Y_{XT}	Mass of cells present per mass of transporters	Mass per mass

$$\int_{X_0}^{X} \frac{dX}{X} = \mu \int_0^t dt \tag{2}$$

gives rise to the half-logarithmic (base 10) plot of log X versus time

$$\mu t = 2.303(\log X - \log X_0) \tag{3}$$

Equation 3 is usually given as a plot of log X on t to evaluate the "lag time" or time interval between inoculation and growth at the characteristic rate, the value of the characteristic time for the population to double in unrestricted batch

TABLE 2. Maximal growth rates and temperature dependencies[a]

Species	Type	Size (μm^3)	μ_{max}		E_a (kcal/mol)	Reference
			h^{-1}	Temp (°C)		
Escherichia coli	Enteric bacterium	0.5	1.97	42	14.2	Ingraham (113)
Pseudomonad 21-3c	Psycrophilic bacterium		1.2	32	9.1	Ingraham (113)
Enterobacter cloacae	Freshwater bacterium	1	0.559	25	67.0	Höfle (105)
Cytophaga sp.	Freshwater bacterium		0.308	25	16.9	Höfle (105)
Cytophaga johnsonae	Freshwater bacterium		0.278	25	12.7	Höfle (105)
Rhodotorula sp.	Marine yeast	36	0.174	18	26.0	McNab (M.S. thesis, University of Alaska, Fairbanks, 1969)
Pseudomonas fluorescens	Freshwater bacterium	1	0.131	10	60.6	Höfle (105)
Thalassiosira weisflogii	Marine diatom	1,100	0.075	20	24.5	Goldman and Glibert (84)
Phaeodactylum tricornutum	Marine diatom	120	0.066	20	10.8	Goldman and Glibert (84)
Unaliella ertiolecta	Marine chlorophyte	311	0.058	20	21.3	Goldman and Glibert (84)
Chaetoceros simplex	Marine diatom	98	0.058	20	11.6	Goldman and Glibert (84)

[a] Arranged in order of decreasing growth rate.

culture t_d, and the final population that can be obtained in the medium supplied.

The exponential form of equation 3 is

$$X = X_0 e^{\mu t} \quad (4)$$

and the time for the population to double is

$$t_d = \ln 2/\mu \quad (5)$$

If the population were to grow at rate μ but without change in the base biomass, a longer time, τ, would be required for the population to double, which is simply reciprocal specific growth rate

$$\tau = 1/\mu = 1.45\ t_d \quad (6)$$

τ is sometimes referred to as the birth time or interval that an organism must exist before dividing when in a population that has a particular growth rate μ.

During growth with ample water and nutrients, the resulting growth rate is an inherent characteristic of that organism, μ_{max}, subject to modification by nutrient balance and culture history (34). Growth proceeds at a maximal rate that increases with temperature until functions are impaired. The change in μ_{max} with temperature is sometimes expressed as a Q_{10}, the temperature rise that causes the rate to double. Alternatively, the change is expressed as an activation energy, E_a, which comes from transition state theory for reversible catalized reactions and the assumption that maximal growth rate is set as if it were determined by a single rate-limiting step. The relationship is

$$\mu_{max} = A \exp(E_a/RT) \quad (7)$$

Maximal growth rates are used to characterize the specific ability of populations to increase under favorable conditions and also to identify the growth rate ($\mu_{max}/2$) that Michaelis concentrations specify. Interpretation of E_a values are complicated by multiple interactive phenomena such as a sharp change in membrane properties at the transition temperature. However, they remain a convenient way to demonstrate and express the amount of change in rate with temperature. Typical values and activation energies associated with various organisms are given in Table 2.

SUBSTRATE UPTAKE

Growth is the direct result of substrate uptake. The model of Fig. 1 expresses the tie between uptake and growth with the liberation of waste products as the energy of chemical bonds is harvested. Sometimes this energy comes from light or chemoautotrophic processes as well. The rate of substrate uptake, v, of a culture depends directly on its biomass. If the concentration of limiting substrate is low ($A_{out} < K_t$), rate also depends on the concentration of substrate (32, 33)

$$v = -\frac{dA_{out}}{dt} = a_A{}^0\, X\, A_{out} \quad (8)$$

according to the kinetics of a catalyzed second-order reaction. The rate-determining second-order rate constants are incorporated into the specific affinity $a_A{}^0$(33) to account for the fact that various parallel transport pathways may exist and further that the organisms are comprised of variable amounts of transporter, permease, enzyme at the rate-

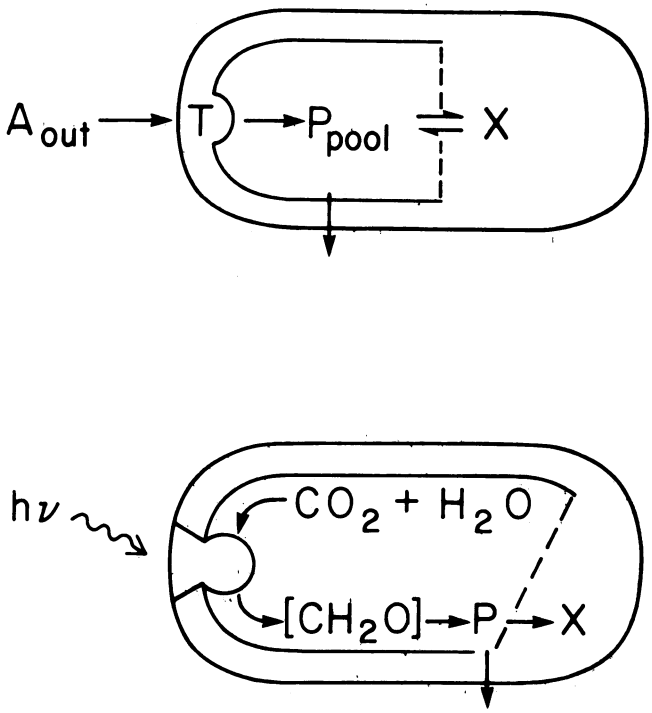

FIG. 1. Models for the active transport of substrate. See text.

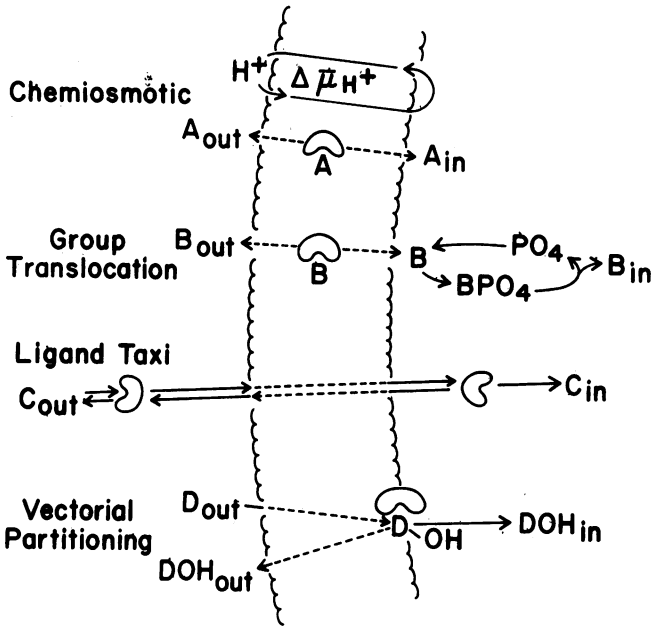

FIG. 2. General models for dissolved nutrient A_{out}-limited growth (top) and light-limited growth (bottom). (Top) Transporter T aids accumulation of nutrient in pools where conversion to products P and cell material X is mediated. (Bottom) Light energy is converted to chemical bond energy and used to form carbohydrate, cell material, and other products. Some of the products escape from the cells in both systems.

limiting step, and other materials which change with conditions (see below).

Transport Mechanisms

Four general models for the active transport of substrates are shown in Fig. 2. The first is the chemiosmotic system which involves an activated membrane or activated water (6) as indicated by $\Delta\bar{\mu}_H{}^+$. A polar substrate such as an amino acid, sugar, or mineral salt contacts a transporter within the cell envelope. The transporter may involve a binding protein (151) and is coupled to an energy source such as adenosine triphosphate, ion gradient, or light by mechanisms not fully understood. The result is high internal concentrations of unmodified substrate (21). Enzymatic conversion of these high substrate concentrations to product then proceeds.

The phosphotransferase system is the best-known example of group translocation (59). Sugars are often transported by this mechanism, which involves phosphorylation and thereby trapping of those substrates which membrane carriers can present to the appropriate phosphorylase (59).

A third, well-documented transport mechanism, but one which is not usually designated as comprising a separate class, is described as ligand "taxi" (65). Specific carriers are exuded by the organism. These carriers bind substrates and then are recollected along with the substrate. Iron is transported in this way by the siderophore of algae (162). Paraffinic hydrocarbons are also thought to be collected by absorbent carriers which are exuded by certain bacteria (121).

Vectorial partitioning is a transport mechanism proposed for substrates that are too nonpolar for the cell membrane to retain. A favorable partition coefficient of lipophilic substrate between phospholipid and water (141) results in amplified hydrocarbon concentrations in the cell membrane. There membrane-bound enzymes take advantage of the high concentrations to produce hydroxylated products (product D-OH of substrate D shown) that are more polar (11, 38). Those which diffuse inward are trapped and can build to high concentrations for subsequent reactions at enhanced rates (D. K. Button, K. S. Craig, and B. R. Robertson, Fed. Proc. 43:2056, 1984).

Rate Constants

For the simplest possible model of single substrate which is involved with a single transporter the rate constants are

$$T + A_{out} \underset{2}{\overset{1}{\rightleftharpoons}} TA \xrightarrow{3} T + A_{pool} \tag{9}$$

Then, usual steady-state assumptions (178) that (i) free transporter T and the transporter-substrate complex TA are constant in time, and (ii) there is correlation of the formation rate of TA with substrate concentration A_{out} and the value of the maximal rate as set by the disassociation rate of TA, gives $K_t = (k_2 + k_3)/k_1$ and $V_{max} = k_3 T_t$. If both back reaction rate K_2 and pool leakage rate $k_5 A_{pool}$ (189) are included in a net rate constant k_{net} (in which k_1 may be the dominant term),

$$\frac{dA_{out}}{dt} = k_{net}\, T\, A_{out} \tag{10}$$

Since total biomass, rather than transporter or enzyme, at the rate-limiting step is the usually measured denominator of microbial activity, it is used as the base for equation 8. Use of surface area would provide certain advantages over biomass, but does not account for activity contributed by internal structures. If the amount of transporter in the organisms is specified as the transporter content of the system divided by biomass,

$$\frac{T}{X} = R_{TX} \tag{11}$$

and the second-order rate constant for transport is taken as $k_{net} = k_1 - k_2$ to account for both back reactions and leakage, then the relationship between the rate constant for transport and the specific affinity of the cell for substrate is

$$a_A{}^0 = k_{net}\, R_{TX} \simeq k_1\, R_{TX} \tag{12}$$

which together with equations 10 and 11 gives equation 8.

In the vectorial partitioning model the concentration of substrate is affected by the partition coefficient ξ for substrate from the aqueous exterior into the lipid portion of the cell membrane. This brings high substrate concentrations to the active sites of membrane-bound enzymes. In this model the size of the partition coefficient and the amount of rate-limiting enzyme at the cell membrane are thought to affect the specific affinity, and

$$a_A{}^0 = k_{net}\, R_{mX}\, R_{Em}\, \xi \tag{13}$$

Light is the nutrient that limits phytoplankton to a near-surface existence. The process (Fig. 1) resembles substrate accumulation by partitioning in that photons are funneled by accessory pigments into a rate-determining photosynthetic center. At that point enzymatic reduction of carbon dioxide occurs. Under light-limited conditions carbon dioxide reduction occurs at a rate that is set by light intensity, by the number and efficiency of photosynthetic centers, and by an upper limit that is characteristic of the organism, conditions, and culture history. The units of specific affinity reflect the

fact that light intensity replaces the substrate concentration of equation 8

$$\begin{array}{lcccccc} \text{photo-} & & \text{specific} & & & & \text{light} \\ \text{synthesis} & = & \text{affinity} & \cdot & \text{biomass} & \cdot & \text{intensity} \\ v & = & a_{h\nu} & \cdot & X & \cdot & I \end{array} \quad (14)$$

$$\frac{\text{g}[CH_2O]}{\text{cm}^3 \cdot \text{h}} \quad \frac{\text{g}[CH_2O] \cdot \text{cm}^2}{\text{g of cells} \cdot \mu\text{Einstein}} \quad \frac{\text{g of cells}}{\text{cm}^3} \quad \frac{\mu\text{Einsteins}}{\text{cm}^2 \cdot \text{h}}$$

The specific affinity reflects the amount of the rate-limiting component that sets the rate of carbon dioxide reduction and the efficiency of the cells in harvesting light for that photosynthetic conversion. The process is complicated over long periods by fluctuation in light intensity in that yields of biomass from carbon dioxide fixed reflect periodic dominance of dark reactions.

The difference between the units of specific affinity in equations 8 and 14 depends on the type of limitation. For photosynthesis in moderately deep aquatic systems, carbon dioxide is usually in excess and rate is often limited by light intensity. The mass of cells and CH_2O could be reported in terms of carbon which cancel one another to simplify units. However, expression of the specific affinity as grams of $[CH_2O]$ per square centimeter per gram of cells per micro-Einstein reflects the delivery rate of the first quantifiable product of photosynthesis (adenosine triphosphate is an earlier but unstable product [166]) into a particular mass of cells as a function of light intensity. In active transport there is often sufficient energy devoted to energizing the cell membrane so that metabolic pools are maintained, and substrate concentration limits rate. For CO_2 limitation, equation 14 takes the form of equation 8. For limitation of transport by supply of energy to transporters in the cell membrane from chemoautotrophy, equation 8 also applies; limiting substrates such as iron and sulfur compounds supply electrons and the yield thus reflects the harvest of energy rather than material and equation 8 takes an energy-dependent form of the type given by equation 14.

Kinetics Based on Continuous Culture

Numerous observations of growth rate controlling nutrient concentrations, adaptive strategies, and uptake kinetics by nutrient-limited organisms have been based on continuous culture. The system inherently strives for a steady state which allows a growth rate fast enough to prevent complete dilution of the culture by fresh medium but slow enough to allow replenishment of used nutrient so that growth can proceed. Thus growth rate is set by the rate of flow of limiting nutrient from the feed into the culture vessel, and biomass is set independently by the amount of limiting nutrient in the feed.

The basic formulations (36, 102) derive from a material balance in the culture vessel or reactor where

$$\begin{array}{l} \text{change in biomass} \\ \text{with fresh medium} \end{array} = \text{growth in reactor} - \begin{array}{l} \text{loss by dilution} \\ \text{with fresh medium} \end{array}$$

$$V\frac{dX}{dt} = \mu XV - FX \quad (15)$$

Dividing through by reactor volume V and substituting the dilution rate $D = F/V$ for a perfectly mixed system (by definition) gives the growth rate in terms of the feed rate, reactor volume, and dilution rate. For the transient state, integration between initial population X_0 at $t = 0$ and X at $t = t$ gives

$$\mu = D + \frac{1}{t}\ln\frac{X}{X_0} \quad (16)$$

As the system arrives at steady state with a balance between growth and washout, $X = X_0$ and

$$\mu = D \quad (17)$$

Limiting nutrient is distributed among organisms, metabolic products, and intercellular substrate. The material balance is

$$\begin{array}{l} \text{change in} \\ \text{substrate} \\ \text{concentration} \end{array} = \text{input from feed} - \text{output by dilution} - \begin{array}{l} \text{substrate} \\ \text{retained} \\ \text{by cells} \end{array}$$

$$V\frac{dA}{dt} = FA_0 - FA_{out} - \frac{\mu XV}{Y_{XA}} \quad (18)$$

At steady state where $dA/dt = 0$, dividing by equation 17 gives

$$X = Y_{XA}(A_0 - A_{out}) \quad (19)$$

Rate, Biomass, and Yield

Direct dependency of substrate uptake rate v on population has been established in continuous culture (36) and by other techniques. Thus culture population depends directly on influent substrate concentration A_0 according to equation 19.

Departure from equation 19 due to the cycling of leaked pool constituents can be demonstrated mathematically to affect A_{out} through change in biomass (unpublished data). But effects are small except where systems are very dilute and are therefore not included in formulations. The rate of growth at steady state is then given by equation 17. Growth kinetics can be determined from appropriate observations according to equations 17 and 19 together with assumptions of perfect mixing and complete viability of the biomass. The relation between biomass concentration and influent nutrient concentration (equation 19) should hold if the biomass is sufficiently high to be unaffected by dilute unintended toxic constituents of the medium, low enough to avoid limitation by some other nutrient B and also to avoid the buildup of inhibitory metabolic products, and if the dilution rate is adjusted to between $D = \mu_{max}$ and some characteristic minimum value (34, 159). The substrate consumed to maintain a particular growth rate depends on the yield of cells produced from substrate utilized Y_{XA} at the growth rate in question

$$\frac{dX}{-dA_{out}} = Y_{XA} \quad (20)$$

The value of the yield is variable. Endogenous metabolism can cause cell yields from oxygen and carbon sources to decrease with decreasing growth rate (see below). Yield may also decrease with decreasing growth rate due to economizing of structural components. In the case of phosphate limitation, cells can be unusually large (24), probably as a result of conservation of phospholipid in cell membranes. The high nutrient concentrations ($A_{out} > K_\mu$) that support fast growth rates can stimulate the formation of nutrient storage compounds which can appear as polyphosphate for phosphate (181), various glycosides for carbon (171), thick

tests for silicon (206), and cyanophycin for nitrogen (163). Control mechanisms lead to the synthesis of additional enzymes, such as hydroxylases in the case of hydrocarbon exposure (11), or repression of others, such as photosynthetic systems in the case of excess light (8, 41).

Saturation

Nutrient uptake rates can display elements of saturation kinetics because enzyme- or transporter-dependent steps limit nutrient flux into the cell. The analogy between the kinetics of transporter-dependent active transport and the kinetics of enzymatic reactions is widely drawn (52). Calculations that use simple models demonstrate that reaction kinetics, both upstream and down from the rate-limiting or "bottleneck" step, have influence on the flux at the rate-limiting step. However, this influence is small and nutrient flux is set in large measure by the "flux-generating enzyme" (164), that is, the step that is slow (180). Therefore, transport kinetics, like complex enzymatic reactions, still follow the Michaelis-Menton equation. These kinetics derive from the simple model described by equation 9 (178):

$$v = \frac{-dA_{out}}{dt} = \frac{V_{max}\, A_{out}}{K_t + A_{out}} \tag{21}$$

The utility of equation 21 is demonstrated by the numerous published good fits of transport data to it, usually presented as one of the linear transformations (108). These data do not, however, speak to the validity of equation 9 from which equation 21 derives, because the rate of increase of many biological reactions seems to fall away with concentration for reasons in addition to saturation and give equally good fits to the equation of Michaelis and Menton.

Kinetics of Growth from Nutrient Uptake

The specific growth rate μ depends on the total substrate uptake rate v per unit population X and the yield of cells formed from substrate consumed Y_{XA},

$$\mu = \frac{v\, Y_{XA}}{X} \tag{22}$$

Substituting v from equation 21 gives the yield- and transport constant-dependent expression for growth

$$\mu = \frac{V_{max}\, Y_{XA}}{X}\left(\frac{A_{out}}{K_t + A_{out}}\right) \quad 0 < \mu < \mu_{max} \tag{23}$$

which holds for values between μ and μ_{max} if the relationship between yield and growth rate is incorporated into Y_{XA} (as is seldom done). If the maximal specific growth rate μ_{max} is set equal to the maximal specific uptake rate V^S_{max} (= $V_{max}Y_{XA}/X$), so that $\mu_{max} = V^S_{max}\, Y_{XA}$, which can be the case during growth limitation at μ_{max} by the transporter content of the cells, substitution into equation 23 gives

$$\mu = \frac{\mu_{max}\, A_{out}}{K_t + A_{out}} \tag{24}$$

Since Y_{XA} is dependent on μ which, in turn, is incorporated into K_t, the nature of the μ-on-A_{out} plot depends on K_t and Y_{XA}. This is a further departure from enzyme and transport kinetics because, in the theory for either, the catalyst content of the system (E_t/X or T_t/X) is usually held constant. The change in Y_{XA} sometimes causes additional curvature in the μ-versus-A_{out} plot, a change which can allow it to take the form of the Michaelis-Menton equation even when K_t exceeds the concentration at half-maximal growth rate K_μ (see below). This saturation- and yield-dependent shape has led to the Monod equation for growth

$$\mu = \frac{\mu_{max}\, A_{out}}{K_\mu + A_{out}} \tag{25}$$

It is obtained from equation 24 by substituting K_μ for K_t (even though K_μ and K_t are equal only when transport limits maximal growth rate). Thus, in agreement with Droop et al. (62), equations 24 and 25 are entirely empirical in nature.

KINETIC CONSTANTS

V_{max} and K_t

Maximal uptake rate V_{max} is the initial rate of substrate uptake at saturating concentrations. It is usually thought to depend on the amount, activity, and sequestering ability of transporters and is therefore affected by induction (184). Since transport capacity can exceed growth requirements at saturation, activity is also thought to be under metabolic control in many situations (46, 58, 63, 106, 189, 195). For example, maximal uptake values of ammonia for *Phaedactylum tricornutum* increased by a factor of 130 in response to increased limitation due to NH_4^+ and shortening the observation time to 15 s (85). Caperon (39) mentioned this problem during early studies of nitrate limitation. Thus highly active transport systems can also fill metabolic pools quickly, which complicates rate determinations by necessitating measurements over very short time intervals. Nutrient transport capacity can be turned off as well as on in response to increased nutrient. Nitrate uptake by batch-grown phytoplankton, for example, is lowest before the resource is depleted. After nitrate runs out the nitrate transport capacity of harvested cells is enhanced (220). In addition, maximal rates often depend strongly on pH, particularly for ionic substrates such as phosphate because of coupling with proton flow (92).

Sometimes V_{max} is normalized to a standard number of cells (213) or to the number of individual cells (68). However, since both the inter- and intraspecies sizes of microorganisms vary, resulting kinetic parameters are difficult to interpret. V_{max} is also reported as micromoles per liter per hour and micromoles per hour (114) which can be converted to specific rates only if the biomass of the particular observation is given. Often uptake is reported as a maximum specific (per unit biomass) rate designated V^S_{max} here, or in units generally reflective of biomass such as dry weight, protein, or carbon.

K_t is variously identified as the Michaelis constant for transport, the saturation constant, or the transport constant. As for complex enzyme systems, K_t is simply taken as the limiting nutrient concentration at $V^S_{max}/2$. Thinking of V^S_{max} and K_t in terms of rate constants (see equations 10 and 13) helps to focus attention on the mechanics of transport. However, mechanisms are insufficiently clear at this time for giving transport constants in detailed rate constant form. Since K_t is related to V^S_{max}, if the value of the maximal uptake rate is preferentially depressed at high nutrient concentration due to a changing cell quota, then the curve of uptake rate on concentration will prematurely bend down, giving an underestimate of K_t.

The non-Michaelian transport kinetics frequently observed are usually explained as due to multiple transport systems operating in concert (4, 29, 57, 194, 202, 211); indeed, genetic evidence supports the fact that such can

occur (194). The two systems are usually described as a high-affinity (low K_t)–low-V_{max} system plus a low-affinity (high K_t)–high-V_{max} system. Combination of these systems is reflected by less curvature in the v-versus-A_{out} plots (33). Organisms associated with a low K_t are thought to have a special advantage in low-substrate environments (98). According to the mobile carrier hypothesis (90), membrane-soluble ligands shuttle material across the membrane; at least the concerted action of several somewhat mobile proteins is probably involved, often in combination with cotransport of a small ion. The effect on transport formulations such as equation 10 is to increase the lifetime of TA. As the residence time of substrate on transporter increases, k_3 decreases, lowering V^S_{max} ($=k_3T_t$ analogous to k_3E_t of enzyme kinetics); additional time therefore becomes available for replenishment of A at the potential site for free T to appear at the membrane surface. One consequence of a slow process is to lower the observed bulk concentration at transport rate $V^S_{max}/2$, thereby lowering K_t. Thus a possible explanation of small K_t values is slow transport systems that expose empty active sites to substrate for only brief periods and the small K_t is due to a small V^S_{max}.

The maximal transport rate V^S_{max} can be increased independently of an organism by simply producing more transporters, which also increases the specific affinity. Maximal velocity from equation 10, k_3T_t, has units of grams of substrate per gram of transporter per hour; however, in the derivation of equation 21 rate v is compared to free enzyme (transporter) $v/V_{max} = T/T_t$ and units for v and V_{max} cancel to hours^{-1}. This has led to difficulties in transport and growth kinetics because transport rate at a given concentration is directly dependent on T and therefore T_t, a phenomenon not reflected by V_{max}, or by K_t which depends on V_{max}. However, if V^S_{max} is reported, as it sometimes is (33, 98), in units of substrate per unit time, this problem is eliminated.

μ_{max} and K_μ

Maximal growth rate, μ_{max}, is a specific characteristic of all organisms. It is related to their ability to reproduce in nutritional conditions of plenty. The Michaelis constant for growth, then, is the substrate concentration where organisms are substrate limited to a growth rate of half the prevailing maximal value. Because of the adaptive ability of organisms, both μ_{max} and K_μ can vary with the nature of the growth medium and the duration of exposure (adaption time) to it. For example, by gradually increasing the dilution rate over a period of a few weeks of a continuous culture in which a marine yeast was growing, μ_{max} could be doubled (34). A similar increase in μ_{max} was noted for both silicate- and ammonium-limited continuous cultures of *Skeletonema costatum* (95).

If transport capacity limits the rate of growth, then K_μ and K_t are similar. However, a common consequence of induction is an increase in the organism's content of transport enzymes for inducing substrates as observed for sugars (184), amino acids (50), and hydrocarbons (11). Conversely, deprivation of an essential nutrient can also result in increased synthesis of the accumulating system. For example, as light is restricted, chlorophyll *a* increases in *Pyrocystis noctiluca*; the specific rate of increase is 0.33 day^{-1} (186). Chlorophyll also increases at low light intensity in *Gonyaulax polyedra* but the organism switches to growth from stored carbon reserves for a time. If light returns, reserves are replenished before growth commences, allowing the cells to survive an additional cycle of light-deficient (deep-water) conditions. For organisms that generate high affinity by synthesizing large amounts of transporter, nutrient transport must be shut down in times of plenty to avoid unusual cellular chemistry. Apparently phosphate transport can be so regulated (189). Uptake capacity for a nutrient is sometimes reduced in preference to an alternative form either by limiting transporter activity or by the activity of an enzyme downstream. Thus, V^S_{max} for nitrate is reduced in regions of the ocean high in ammonia (146).

K_t and K_μ

The specific affinity of an organism for a particular substrate can be increased by synthesizing transport enzymes to high density within the organism (33). V^S_{max} is increased in direct proportion to this added capacity, but μ_{max}, a specific characteristic, is unaffected. If the new transport material is of constant type, then K_t is unaffected but K_μ is reduced consistent with new ability to sequester nutrient at low concentration.

Another difference between K_t and K_μ then stems from the fact that yield may be a very strong function of growth rate (see below). Analysis of the kinetics of phosphate-limited transport and growth, for example, showed that excess phosphate transport capacity was generated to allow growth at low phosphate concentrations which would have given a linear relationship between phosphate concentration and growth at constant yield. However, the lower yield of phosphate-rich cells at high growth rates caused curvature, which gave a good fit to equation 25 (189).

Change in yield may also have subtle effects on the interpretation of adaptive strategies. Sometimes rates of nutrient uptake v_A are normalized to the amount of nutrient incorporated (40, 48, 63, 83, 96). The nutrient concentrations cancel, giving units of hours^{-1}. These are the same units as used in enzyme kinetics to express a rate which is pseudo-first order with respect to substrate concentration. But the value depends on the nutrient content of the cells. Cells exposed to high nutrient concentrations develop high nutrient contents or cell quotas for common bioelements such as nitrogen, phosphorus, silicon, and coenzymes such as vitamin B_{12} (62). Thus, during the exposure of organisms to the large nutrient concentrations required in a perturbation experiment, the progressively depressed rate of nutrient uptake observed may be due in part to use of progressively smaller quantities of cell material as a base for the rate determination: a changing base due to declining yield. This gives premature curvature to v/A_{out} plots from the point of view of saturation kinetics. If the activity of transporters is regulated downward by high nutrient levels during the course of the experiment, the value of K_t is further depressed. Thus curvature in both growth rate (see above) and uptake rate versus substrate concentrations can result from use of a sliding base with additional complications due to change in transporter activity.

Affinity

As applied to the collection of substrate by microorganisms, affinity is formulated in various ways (Table 3). The term was first used (197) by Guldberg (88) to express the hydrolytic combination of acid with sucrose which led to the law of mass action. For enzymes affinity was taken as k_1/k_2 (equation 10) to describe a system at static equilibrium with constant concentrations of substrate and product (101) and later extended by Haldane (see reference 197) to dynamic systems where there is a steady rate of catalysis at thermo-

TABLE 3. Terms used to express substrate collection ability for transport and growth

Term	Symbol	Unit(s)	Transport equation	Reference
Affinité	S_k	Moles per liter	$\mu = \frac{\mu_{max} A}{K_\mu + A_{out}}$	Monod (158)
Affinity	$1/K$	Liters per mole of A	$v = \frac{V_{max} A_{out}}{K_t + A_{out}}$	Cuhel et al. (51)
Second-order rate constant	k	Liters per organism per h	$v = \left(\frac{\mu_{max}}{Y K_\mu}\right) A_{out} X_N = k A_{out} X_N$	Paris et al. (170), Rickenberg et al. (184)
Pseudo-first-order rate constant for transport at constant cell population	k	Hours^{-1}	$v = \left(\frac{\partial A_{out}}{\partial t}\right) X = k A_{out}$	Button et al. (38)
Affinitätskonstant	k	Hours^{-1}	$v = \frac{\Delta A_{out}}{\Delta t} = k A_{out}$	Koschel (129)
Transport efficiency	E_t	Seconds^{-1}	$v^S = \frac{V^S_{max} A_{out}}{K_t}$ as $A_{out} \rightarrow 0$ $E_t = \frac{V^S_{max} X}{K_t}$	Koch (126)
Growth efficiency	E_g	Seconds^{-1}	$E_g = \frac{\mu_{max} A_{out}}{Y_{XA} K_\mu}$	Koch (126)
Initial slope[a]	$\frac{\Delta P'}{\Delta I}$	Grams of C per gram of chlorophyll chl *a* per kilolux second	$v = \frac{\Delta P'}{\Delta I}$	Parsons and Takahashi (171)
Initial slope	$\left(\frac{\partial \mu}{\partial \xi}\right)_0$	Square meters per gram of chlorophyll *a* per hour	$\mu = \left(\frac{\partial \mu}{\partial \xi}\right)_0 k_c I$	Laws and Bannister (138)
Specific affinity	$a_{h\nu}$	Grams of [CH_2O] per square centimeter per gram of cells per microEinstein	$v = a_{h\nu} X I$	This communication
Specific affinity	$a_A{}^0$	Liters per gram of cells per hour	$v = a_A{}^0 X A_{out}$, a_A as $a_{out} \rightarrow 0$	Law and Button (135)
	$\frac{V^S_{max}}{k_t}$	Liters per micromole of A_{out} per gram (dry) of cells per hour	$v = \frac{V^S_{max} A_{out}}{K_t}$	Healey (98)
Affinity	α_μ	Liters per gram of cell *A* per hour	$v = \frac{V_{max} A_{out}}{K_t + A_{out}}$	Goldman and Glibert (*in Nitrogen in the Marine Environment*, in press)
Affinity[b]	α_μ	Liters per gram of cells per hour	$v = \frac{\mu_{max} A_{out}}{K_\mu + A_{out}}$	Goldman and Glibert (*in Nitrogen in the Marine Environment*, in press)
Specific affinity[c]	$a^{[A]}{}_A$	Liters per gram of cells per hour	$v = a_A{}^0 X A_{out}$, v/A_{out} as A_{out}	Button (33)

[a] Slope breaks sharply to zero at $I = P'_{max}$.
[b] Complete units are: grams of cells formed per liter per gram of cells present · gram of substrate present.
[c] Complete units are: grams of substrate taken up per liter per gram of cells present per gram of substrate present per hour; value of $a_A{}^{[A]}$ approaches $a_A{}^0$ as A_{out} approaches zero.

dynamic equilibrium, using $k_1/k_2 + k_3$. These concepts were directly transferred to transport by Monod (158) and coworkers (184) who spoke of organism substrate "affinité." Koschel (129) normalized uptake rates to substrate concentration and used the resulting first-order rate constant in biomass, which he called the "Affinitätskonstant" as an indication of the relative activity of various water samples. Sometimes rates are compared in systems of constant biomass (34) and k is designated the "pseudo-first-order" rate constant as an index of organism activity.

Affinities have been defined as "second-order rate constants" and given on a per-cell basis by Paris et al. (170). This improves kinetic descriptions of aquatic processes but requires organism size or else invariant bioelement content to compare the kinetics of nutrient uptake by different species with one another. Koch and Wang (127) used rates in

terms of per unit of biomass to calculate transport efficiencies and growth efficiencies. These terms appear to be biomass dependent.

The photosynthetic efficiency of phytoplankton has been reported in terms of the "initial slope" of the photosynthesis-versus-light intensity curve which compares the rate of fixation of carbon per unit of chlorophyll to light intensity by Parsons and Takahashi (171) and by Platt and Jassby (177), which stems from the early work of J. F. Talling (Ph.D. thesis, University of Leeds, Leeds, U.K.). This idea is reexpressed by equation 14 in terms of quanta received by the cells and is based on cell mass rather than chlorophyll because accessory pigments can also help to funnel in photons (169). In either case P'_{max} is a useful term which defines the light intensity where, in case of excess photosynthetic capacity, needs of the organism are satisfied and additional photosynthesis is not required as light continues to increase. Droop et al. (62) evaluated light limitation in terms of light absorbed per unit of biomass. Biomass was given in terms of joules per liter and corrected for dark respiration so that the harvest of electromagnetic energy is nicely defined and units are simplified. However, in this, as in much published kinetic work on microbial processes, the common denominator of biomass is cell number so that calculation of specific affinities from the data requires assumptions about the mass per cell. Light intensity at the intersection of the initial slope and the photosynthetic rate at saturation is sometimes designated I_k (207), a concept that could apply equally well to nutrient uptake systems where excess transport capacity exists and has the attraction of not specifying Michaelian or other kinetics.

Healey (98) advocated use of V^S_{max}/K_t, called simply V/K, for comparing the nutrient sequestering ability of phytoplankton. This relatively useful term suffers from the fact that Michaelian kinetics are inherently specified and the value of V^S_{max} must be obtainable. For systems with much excess transport V^S_{max} is difficult to obtain experimentally (189). For organisms with multiple transport systems the form of the Michaelian relationship, equation 21, is inherently slightly incorrect (33).

Affinity is generally regarded as the attractive force between two particles. For a species of transporter content VX/T_{XA}, that force results in a transport rate $k_{net}XA_{out}R_{TX}$ which is from equations 8, 11, and 12 in the form of a transporter-dependent second-order rate equation. Thus, the specific affinity has been defined as $a_A{}^0$, the initial slope of the V^S-versus-A_{out} curve which, for a transport system described by equation 9, is related to the mechanism by $a_A = k_{net}/Y_{XT}$, where $k_{net} = k_1 - k_2$ and Y_{XT} gives the concentration of transporters in the organism (33). As with enzymatic reactions, the maximum probability for transport of a molecule of A occurs at low substrate concentrations. Then, as A_{out} approaches zero, the specific uptake rate v^S approaches the expected rate predicted by the derivative of equation 21, and $a_A{}^0 = V^S_{max}/K_t$. The value of the specific affinity in equation 8 is therefore V_{max}/K_t for the ideal hyperbolic system formulated by equation 21. The value of the specific affinity decreases to progressively lower values, a_A ($= v^S/A_{out}$), at higher substrate concentrations as saturation becomes significant (Fig. 3). Thus no particular kinetics are specified for the relationship between transport rate and A_{out}; a_A depends only on the value of v^S at the value of A_{out} in question. As saturating concentrations [A] of A are approached, the resulting values of $a_A{}^{[A]}$ ($= v^S/A_{out}$) must be expressed. For example, in the case of a perfectly Michaelian system, $a_AK_t = a_A{}^0/2$. Thus values of the specific affinity at concentrations below the Michaelis constant, and particularly $a_A{}^0$, are useful for comparing the ability of organisms to accumulate substrate and grow at small concentrations. This can be done without knowledge of the organism size, Michaelis concentration, μ_{max}, or particularly V_{max}, which may be indeterminate.

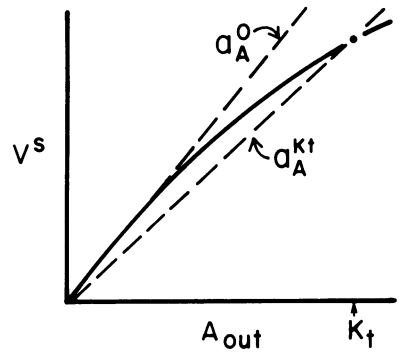

FIG. 3. Kinetic curve showing rate of transport of nutrient A on a per unit of cell mass basis versus concentration of limiting nutrient. The value of the specific affinity is shown both at subsaturating substrate concentrations as given by the initial slope and at the Michaelis concentration where its value is reduced to V^S/K_t.

NON-GROWTH PHENOMENA

Endogenous Metabolism

Even starving cells maintain metabolic pools, form new ribonucleic acid, and make protein (149). The energy costs of these processes are attributed to endogenous metabolism. These endogenous costs affect the yield of cells formed from substrate used. The relationship between the rates of cell production and substrate consumption is set by the yield of cells from substrate at the specific growth rate μ ($= dX/Xdt$) involved

$$\frac{dX}{dt} = \frac{-dA_{out}}{dt} Y_{XA} \tag{26}$$

For heterotrophic growth from organic substrates, part of the carbon source is used for structural material and part is used for an anabolic energy source. Transport and anabolism requirements have been estimated at 0.12 to 0.16 mol of adenosine triphosphate per g of cells (204). Rebuilding and repair functions, leakage, and the maintenance of energized membranes incur additional endogenous or maintenance energy costs. These structural, anabolic, and endogenous requirements are reflected in the value of the yield constant.

Continuous culture is often used to evaluate endogenous costs because growth rates can be controlled independent of substrate concentration in the influent medium. Assumptions are that total substrate cost depends on (i) that for structural material and its biosynthesis as set by the biomass and by the growth yield Y_g, and (ii) additional endogenous costs, costs which are constant with time and depend only on the biomass and the rate constant for endogenous metabolism k_e (150, 175; Button, Ph.D. thesis, University of Wisconsin, Madison, 1964). Thus

$$\frac{dA_{out}}{dt} = \frac{X}{Y_g} + k_eX \tag{27}$$

TABLE 4. Endogenous metabolism

Substrate	Organism	Method	K_e (g/g of cells per h)	Reference
Glucose	*Escherichia coli*	Yield change in continuous culture	0.018	Marr et al. (150)
Glucose	*Pseudomonas* sp. strain 95A	Same	0.013	Jones and Rhodes-Roberts (120)
Glucose	*Pseudomonas* sp. strain 41	Same	0.012	Jones and Rhodes-Roberts (120)
Glucose	*Escherichia coli*	Same	0.015–0.25	Jones and Rhodes-Roberts (120)
Oxygen	*Aerobacter aerogenes*	Oxygen consumption in continuous culture extrapolated to $D = 0$	0.03	Herbert (102)
Oxygen	*Torulopsis utilis*	Oxygen consumption in continuous culture extrapolated to $D = 0$	0.017	Button and Garver (36)
Glucose	*Aerobacter cloacae*	Change in yield in continuous culture, anaerobic	0.014	Pirt (174)
Glucose	*Arthrobacter crystallopoites*	CO_2 production during starvation	0.009	Boylen and Ensign (20)
Glucose	*Spirillum* sp.	Change in yield in continuous culture	0.006	Matin and Veldkamp (152)
Glucose	*Aeromonas hydrophilia*	Minimum substrate addition giving growth	0.0045	van der Kooij et al. (214)
Glucose	*Candida parapsilosis*	Change in yield in continuous culture	0.0038	Rogers and Stewart (190)
Glycerol	*Aerobacter cloacae*	Change in yield in continuous culture, aerobic	0.0028[a]	Pirt (174)
Toluene	*Pseudomonas* sp. strain T2	Oxygen consumption of washed cells after 30 h	0.002[b]	Robertson and Button (unpublished data)

[a] Grams of glucose.
[b] Grams of oxygen.

The endogenous metabolism rate constant is determined by combining equations 15, 16, and 19 to obtain the linear relationship

$$\frac{1}{D} = \frac{1}{k_e}\left(\frac{1}{Y_{XA}}\right) - \frac{1}{Y_g k_e} \tag{28}$$

This endogenous metabolism rate constant can be evaluated from dilution rate and yield data if cell death rates are negligible. Typical values of the rate constant for endogenous metabolism are given in Table 4. This "constant" as defined is empirical in nature and has no particular basis in theory; accordingly, formulations have been recently refined by Pirt (176). However, the values are useful in a practical sense particularly when considering cell production in slowly growing systems.

Respiratory Loss

Phytoplankton subsist on stored carbon during darkness, resulting in added respiratory loss. This loss has been evaluated from the change in the population of *Monochrysis lutheri* in continuous culture during a dark cycle (139). It was assumed that growth during dark was zero and leakage was negligible so that the change in the carbon content (Q_C') with time is

$$Q_C'X = Q_C'X_0 e^{-(D + k_L)t} \tag{29}$$

where k_L is the total rate of carbon loss and k_r is respiratory loss component of k_L. Since loss due to dilution D is known, the remaining loss rate k_r can be calculated. Respiratory loss, as computed at a series of dilution rates, was found to increase in a linear fashion with growth rate ($\mu = D$ at steady state), giving a good fit to the empirical relationship and associated constants

$$k_r = 0.0034 + 0.018\,D \tag{30}$$

Substrate Thresholds

Concentrations of substrate below which cells cannot grow are called threshold concentrations, A_t (32, 40). Endogenous requirements of the organisms for substrate are viewed as the main cause of thresholds for growth. Thresholds are also a consequence of saturation phenomena in certain types of branched biomolecular enzymatic reactions (142). The size of thresholds due to endogenous costs can be estimated from endogenous requirements and specific affinity values. At zero growth rate the substrate consumption is entirely endogenous and

$$\frac{-dA_{out}}{dt} = k_e X = a_A\,X\,A_t \tag{31}$$

where A_t is the threshold for growth, i.e., the value of A_{out} as μ approaches zero. Solving for A_t at steady state where input substrate in continuous culture is equal to the uptake rate v (equation 8) of the cells at steady state and $dA_{out}/dt = 0$,

$$A_t = k_e/a_A \tag{32}$$

For example, at typical values of $k_e = 0.01$ g of glucose/g of cells per h and $a_A = 100$ liters/g of cells per h, $A_t = 0.1$ mg of glucose per liter. Some threshold values are shown in Table 5.

Substrate thresholds are sometimes incorporated into kinetic expressions for growth (32, 40). By defining the effective concentration A^e_{out} as the substrate used for growth over and above endogenous requirements

$$A^e_{out} = A_{out} - A_t \quad (33)$$

growth rate is given from maximal uptake rate, K_t, and the yield (which changes with μ) by the expression

$$\mu = \frac{V^S_{max} A^e_{out} Y_{XA}}{(K_t - A_t) + A^e_{out}}, \quad 0 < \mu < \mu_{max} \quad (34)$$

which is a refinement of the empirical equation 23 for relating growth rate to substrate concentration at low values.

CELL QUOTA

Mackereth (147) and Gerloff and Skoog (81) noticed that the amount of phosphorus per algal cell decreased with growth rate as it became limiting. These observations gave rise to what is known as the cell quota concept. Because interspecies and chemical comparisons are impossible without knowledge of cell size, the cell quota Q' is sometimes expressed as nutrient per gram of cells (40). Cell quota approaches some minimum value k_Q:

$$\mu = \mu'_{max}\left(1 - \frac{k_Q}{Q'}\right) \quad (35)$$

and μ'_{max} is a constant which is somewhat inflated over the maximal growth rate μ_{max}. When μ is plotted against Q', equation 35 has the same general form as equation 24 (and more closely equation 34, because of the Q' intercept), a coincidence that has probably been partly responsible for widespread usage. Droop (61) in an excellent introspective account of algal growth kinetics cited numerous examples where growth rates, as controlled by phosphate, various nitrogen sources, silicon, iron, and cobalamine, followed equation 35. In the special case where all of the substrate collected is laid down and retained as cell material, then

$$Q' = 1/Y_{XA} \quad (36)$$

and substrate uptake is given by the widely used relationship

$$v = \mu Q' X \quad (37)$$

Equation 37 is appropriate for uptake of substrates when excretion of them is negligible. It is not used for heterotrophic uptake because of the large amounts of carbon lost to carbon dioxide and organic products. However, it appears that significant losses occur for other nutrients as well. For example, Chan and Campbell (44) could account for only 40% of the nitrate-nitrogen taken up as particulate material in studies of freshwater phytoplankton. Leakage of both orthophosphate and organic phosphate has been quantified during phosphate-limited continuous cultures of *Rhodotorula rubra* (189). The point that a linear decrease in yield with growth rate gives a good fit to equation 35 can easily be demonstrated graphically. Sometimes Q' is linear, rather than hyperbolic, with growth rate giving curvature to Y_{XA} versus μ (24). Attempts to relate external concentrations of limiting nutrient to growth rate according to equation 35 seem to involve some rather large steps (61). However, the formulation, when limited to algae, can provide a means to bridge fluctuations in nutrient and light. Algae and cyanobacteria often contain nutrient transport capacity sufficient to supply minerals at a rate exceeding requirements. Transport is under regulation (14, 71) so that more nutrient, particularly nitrogen and phosphate, is stored as growth rate increases. This reserve capacity can be called upon in times of nutrient deficiency (12) to extend growth capability into times of nutrient depletion.

TABLE 5. Threshold concentrations for growth

Substrate	Organism	A_t	Reference
Glucose (with added amino acids)	*Corynebacterium* sp. strain 198	<0.16 nM	Law and Button (135)
Glucose (with added arginine)	*Corynebacterium* sp. strain 198	0.83 μM	Law and Button (135)
Glucose (alone)	*Corynebacterium* sp. strain 198	1.3 μM	Law and Button (135)
Lactose	*Spirillum serpens*	50 μM	Jannasch (115)
Arginine	*Corynebacterium* sp. strain 198	0.53 μM	Law and Button (135)
Glucose	*Rhodotorula rubra*	0.55 μM	Robertson and Button (188)
Phosphate	*Rhodotorula rubra*	3 nM	Button et al. (34)
Phosphate	*Nitzschia actinastroides*	10 nM	Müller (159)
Phosphate	*Selenastrum capricornutum*	15 nM	Brown and Button (24)
Silicate	*Cyclotella meneghiniana*	<0.5 μM	Tilman and Kilham (212)
Silicate	*Asterionella formosa*	0.8 μM	Holm and Armstrong (109)
Silicate	*Skeletonema costatum*	0.3 μM	Paasche (168)
Silicate[a]	*Thalassiosira pseudonana*	0.6 μM	Paasche (168)
Silicate	*Licmophora* sp.	1.3 μM	Paasche (168)
Silicate	*Thalassiosira decipiens*	1.3 μM	Paasche (168)
Silicate	*Ditylum brightwellii*	0.4 μM	Paasche (168)
Light	*Gonyaulax polyedra*	10.8 μEinsteins/cm^2 per h	Rivkin et al. (187)

[a] Threshold is for uptake, not growth.

EFFECTIVE DATA COLLECTION

Conditioning of Cells

The kinetic characteristics of growth depend, in part, on the exposure history of the organisms. Three general regimes for substrate uptake studies are used. The first is based on the observations of Monod (157) who found that the growth rate of a batch culture decreased with exposure to decreasing concentrations of nutrient. Such a system is in transient state if the organisms experienced a different nutrient concentration before the experiment than during it. In batch culture systems, the quantity and quality of the biomass, as well as the concentration of nutrient, change over the course of the observation. Gaudy et al. (80) pointed out that, even during restricted exponential growth, the growth rate at a particular time depends on nutrient concentrations at an earlier time. These effects of changing concentrations are sometimes minimized by extrapolating to initial conditions. However, organisms adapted to one nutrient concentration (e.g., the enzyme-saturating concentrations in culture) are often tested for uptake at another. One method first used by Shehata and Marr (198) to minimize the extent of the transient state is to begin with very low populations of organisms and use them over extended periods.

TABLE 6. Organic carbon

Substrate	Organism	Method and incubation time	$Y_{X/S}$ (g of cells per g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity		
						Liters/g of cells per h	At concn (μM)	
Sugars								
Glucose	*Corynebacterium* sp. strain 198	Glucose oxidase/hexokinase in continuous culture; influent medium contained glucose and arginine in equal quantities			2.2	625	0.8	Law and Button (135)
Glucose	*Corynebacterium* sp. strain 198	Glucose oxidase/hexokinase in continuous culture	0.42		2.6	388	1.2	Law and Button (135)
[^{14}C]glucose	*Cytophaga johnsonae*	Steady-state in continuous culture (D = 0.03 h^{-1}); substrate determined enzymatically		1.5	8.6	440	0	Höfle (106)
[^{14}C]glucose	*Cytophaga johnsonae*	Radioactivity uptake, batch-grown cells; 1 min		42		7.7	0	Höfle (106)
Glucose	*Cytophaga johnsonae*	Glucose concentration (hexokinase) at steady state (D = 0.15 h^{-1}), 1,000–1,360 h after inoculation	1.3			252	3.5	Höfle (107)
[^{14}C]glucose	*Cytophaga johnsonae*	Radioactivity uptake of samples above, 1 min				43	1	Höfle (107)
Glucose	*Cytophaga johnsonae*	Glucose concentration (hexokinase) at steady state (D = 0.15 h^{-1}), 40–190 h after inoculation	1.3			5.1	122	Höfle (107)
[^{14}C]glucose	*Cytophaga johnsonae*	Radioactivity uptake of samples above, 1 min				1.8	1	Höfle (107)
[^{14}C]methanol	*Pseudomonas* sp.	Radioactivity uptake by washed cells, 10 min		4.8		225	0	Bellion et al. (10)
Glucose	*Escherichia coli*	Batch culture growth from standard addition			13	180	0	Koch and Wang (127)
[^{14}C]arabinosyl-β-D galactoside	*Lactobacillus casei*	Radioactivity uptake, 10 min		19		93	0	Chassy and Thompson (45)
[^{14}C]lactulose	*Lactobacillus casei*	Radioactivity uptake, 10 min		24		91	0	Chassy and Thompson (45)
Glucose	*Torulopsis utilis*	Extrapolation of added substrate to zero cell population in continuous culture	0.9		49	85	0	Button and Garver (36)
[^{14}C]lactose	*Lactobacillus casei*	Radioactivity uptake, 10 min		14		75	0	Chassy and Thompson (45)
Glucose	*Streptococcus sanguis*	Continuous culture, hexokinase/glucose-6-phosphate dehydrogenase	0.80		111	52	0	Kemp et al. (123)
Glucose	*Streptococcus sanguis*	Batch growth rate, hexokinase/glucose-6-phosphate dehydrogenase	1.05		327	13.3	0	Kemp et al. (123)
Glucose	*Streptococcus mutans*	Anaerobic batch growth rate, hexokinase/glucose-6-phosphate dehydrogenase	0.77		1,583	6.6	0	Kemp et al. (123)
Glucose	*Streptococcus mutans*	Anaerobic continuous culture, hexokinase/glucose-6-phosphate dehydrogenase	0.84		2,172	3.6	0	Kemp et al. (123)
Ribose	*Thiobacillus* sp. strain A-2	Dialysis rate of ^{14}C-labeled substrate over 5 min		69		9.3	0	Wood and Kelly (218)
Fructose	*Thiobacillus* sp. strain A-2	Dialysis rate of ^{14}C-labeled substrate over 5 min		410		3.8	0	Wood and Kelly (218)
Sucrose	*Thiobacillus* sp. strain A-2	Dialysis rate of ^{14}C-labeled substrate over 5 min	2.9	240		3.6	0	Wood and Kelly (218)
Xylose	*Thiobacillus* sp. strain A-2	Dialysis rate of ^{14}C-labeled substrate over 5 min		2,000		1.4	0	Wood and Kelly (218)

Continued on following page

TABLE 6—*Continued*

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells per g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity		
						Liters/g of cells per h	At concn (μM)	
[^{14}C]glucose	*Streptomyces coelicolor*	Radioactivity uptake, 60 min		6,100		0.016		Hodgson (104)
Amino acids								
[^{14}C]arginine	*Corynebacterium* sp. strain 198	$^{14}CO_2$ from arginase-urease treatment of continuous culture samples	0.57		0.5	1,750	0.4	Law and Button (135)
[^{14}C]alanine	*Rhodospirillum rubrum*	Light-driven anaerobic radioactivity uptake at saturating alanine concentrations, 12 mM Mg^{2+}				2.4	16.5	Zebrower and Loach (219)
[^{14}C]alanine	*Rhodospirillum rubrum*	Light-driven anaerobic radioactivity uptake at saturating alanine concentrations, 2 mM Mg^{2+}		6.5 μEinsteins h^{-1}		1.2	16.5	Zebrower and Loach (219)
[^{14}C]alanine	*Rhodospirillum rubrum*	Light-driven anaerobic radioactivity uptake at saturating alanine concentrations, 2 mM Mg^{2+}				0.48	16.5	Zebrower and Loach (219)
[^{14}C]asparagine	*Saccharomyces cerevisiae*	Radioactivity uptake		350		1.7	0	Gregory et al. (86)
[^{14}C]tyrosine	*Brevibacterium linens*	Initial uptake, 5 min		3.4		1.5		Boyaval et al. (19)
[^{14}C]phenylalanine	*Brevibacterium linens*	Initial uptake, 5 min		25		0.36		Boyaval et al. (19)
[^{14}C]tryptophan	*Brevibacterium linens*	Initial uptake, 5 min		1.8		0.1		Boyaval et al. (19)
[^{14}C]glutamine	*Saccharomyces cerevisiae*	Radioactivity uptake		330		0.82	0	Keenan et al. (122)
[^{14}C]asparagine	*Saccharomyces cerevisiae*	Radioactivity uptake		400		0.63	0	Keenan et al. (122)
[^{14}C]glutamate	*Coxiella burnetii*	Radioactivity uptake, pH 3.5		90.2		0.13	0	Hackstadt and Williams (89)
[^{14}C]glutamate	*Coxiella burnetii*	Radioactivity uptake, pH 4.6		49.4		0.03	0	Hackstadt and Williams (89)
[^{14}C]isoleucine	*Streptococcus thermophilus*	Radioactivity uptake, 1 min		16		0.09	0	Akpemado and Bracquart (1)
[^{14}C]isoleucine	*Streptococcus thermophilus*	Radioactivity uptake, 1 min		13		0.03	0	Akpemado and Bracquart (1)
[^{14}C]valine	*Streptococcus thermophilus*	Radioactivity uptake, 1 min		36		0.02	0	Akpemado and Bracquart (1)
[^{14}C]leucine	*Streptococcus thermophilus*	Radioactivity uptake, 1 min		46		0.01	0	Akpemado and Bracquart (1)
Carbonyls								
[^{14}C]glycerol-3-phosphate	*Escherichia coli*	Radioactivity uptake, 1 min		2		429	0	Schweizer et al. (196)
[^{14}C]succinate	*Rhizobium leguminosarum*	Radioactivity uptake by isolated bacteroids; 2 min		1.5		137	0	Finan et al. (73)
[^{14}C]benzoate	*Pseudomonas putida*	Radioactivity uptake, 1–2 min, 20°C		20		27	0	Thayer and Wheelis (210)
[^{14}C]succinate	*Rhizobium japonicum*	Radioactivity uptake, 5 min		3.8		19	0	McAllister and Lepo (153)
[^{14}C]lactobionic acid	*Lactobacillus casei*	Radioactivity uptake, 10 min		26		80	0	McAllister and Lepo (153)
α[^{14}C]amino-isobutyric acid	*Phlyctochytrium* sp.	Radioactivity uptake of starved cells; reaction stopped in single sample with cold substrate at 3 min; 490 mM Na^+		17		8.2	0	Amon and Arthur (2)
α[^{14}C]amino isobutyric acid	*Phlyctochytrium* sp.	Radioactivity uptake of starved cells; reaction stopped in single sample with cold substrate at 3 min; 22 mM Na^+; osmolarity maintained		143		4.9	0	Amon and Arthur (2)

Continued on following page

TABLE 6—*Continued*

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells per g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity		
						Liters/g of cells per h	At concn (μM)	
[^{14}C]glucuronate	*Erwinia carotovora*	Radioactivity uptake, 10 min		50		6.6	0	Hugouvieux-Cotte-Pattat et al. (111)
[^{14}C]mannitol	*Pseudomonas aeruginosa*	Radioactivity uptake, 5 min				0.075	0.00045	Eisenberg and Phibbs (66)
Hydrocarbons and aromatics								
[^{14}C]toluene	*Pseudomonas putida*	Total ^{14}C-labeled product production, 4 h				500		This laboratory (unpublished data)
[^{14}C]toluene	*Pseudomonas* sp. strain T2	Total ^{14}C-labeled product production, induced cells, 4 h	0.28	0.47		1,064	0	This laboratory (unpublished data)
3-Methylcatechol	*Pseudomonas* sp. strain T2	Oxygen consumption by growing cell suspension		31		1.0	0	This laboratory (unpublished data)
[^{14}C]toluene	*Pseudomonas* sp. strain T2	Total ^{14}C-labeled product production, uninduced cells, 4 h	0.01	0.36		0.5	0	This laboratory (unpublished data)
Methane	Mixed bacteria	Combustion of diffused methane from continuous culture	1.69			401	17	Sheehan (Ph.D. thesis, University of Wisconsin, Madison, 1970)
Methane	*Methylosinus trichosporium*	Oxygen consumption by washed cell suspension in methane solutions as determined by gas chromatography		45		29	0	O'Neill and Wilkinson (167)
[^{14}C]dodecane	*Corynebacterium* sp. strain 198	Radioactivity at steady state in continuous culture				4.4	0.009	Law and Button (135)
Phenol	*Pseudomonas putida*	Substrate removal rate, liquid chromotography; 30–70 h		10		3.9	0.2–10	Paris et al. (170)
Methylphenol	*Pseudomonas putida*	Substrate removal rate, liquid chromatography; 30–70 h		10		2.6	0.2–10	Paris et al. (170)
p-Chlorophenol	*Pseudomonas putida*	Substrate removal rate, liquid chromatography; 30–70 h		10		0.9	0.2–10	Paris et al. (170)

A way of obtaining cells adapted to particular nutrient concentrations for substrate uptake studies is to grow the cells at a specified growth rate (equation 10) in continuous culture, harvest, and test their short-term ability to sequester nutrients from defined concentrations (34, 106). Theory attending resulting initial uptake rates is similar to that of steady-state enzyme kinetics because biomass, like enzyme concentration, is of constant composition, with substrate concentration the single independent variable and rate reflecting a steady flow to products. Organisms adapted to the prevailing conditions can be examined for their ability to incorporate nutrient. Detectably large quantities of nutrient are sometimes injected directly into the reactor and uptake rates are computed from the rate of nutrient decay according to equation 18 (40, 48, 55). If the continuous culture is converted to batch mode by shutting off the pump and therefore feed rate, F, the rate of nutrient uptake at any particular moment and nutrient concentration is

$$v^S = \left(\frac{\partial A_{out}}{\partial t}\right)_X \tag{38}$$

Decrease in concentration of an isotope A^*_{out} injected into the reactor of a continuous culture is given by (adapted from Button and Kinney [37])

$$\frac{dA^*_{out}}{dt} = D\,A^*_{out} + v^*_{net} \tag{39}$$

If the change in population due to an added radioactive substrate is small, the specific affinity of the cells for it can be obtained from the change in radioactivity with time,

$$\ln \frac{A^*_{out}}{A^*_{out\ 0}} = -D(X + a_A X)t \tag{40}$$

TABLE 7. Nitrogen

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells/g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity		
						Liters/g of cells per h	At concn (μM)	
Ammonia	*Skeletonema costatum*	Perturbation analysis of continuous culture; D = 0.04 h^{-1}; NH_3 = 7 μM; kinetics based on particulate N	1,050–4,900	1–2	<0.5	4,051	0	Conway et al. (48)
Ammonia	*Thalassiosira pseudonana*	Continuous culture limited by both ammonia and nitrate; D = 0.017 h^{-1}; uptake by depletion of 10 μM spike	83	0.04	0.28	1,116	0	Eppley and Renger (68)
^{15}N-ammonia	*Thalassiosira pseudonana*	Mass spectrographic analysis of batch-grown cells immediately after nitrate depletion; 5 min				860	10	Horrigan and McCarthy (110)
Ammonia	Phytoplankton of seawater	Isotope dilution, 4 h; remineralization may increase rates by 1/3				182 2.8	0.05 6.6	Garside and Glibert (79)
^{15}N-ammonia	Marine phytoplankton; eutrophic seawater	Mass spectrometry of particulate fraction of ammonia-amended samples, 6 to 24 h		1.3		40	0	MacIsaac and Dugdale (145)
^{15}N-ammonia	As above; oligotrophic seawater	As above		19		19	0	MacIsaac and Dugdale (145)
Ammonia	*Methylosinus trichosporium*	Oxygen consumption by washed cell suspension, Van Slyke method; NH_3 from pH		10		7.4	0	O'Neill and Wilkinson (167)
Ammonia	*Nitrosomonas europaea*	Oxygen uptake of centrifuged cells from batch culture; ammonia calculated from NH_4^+ and pH		50		0.045	50	Hyman and Wood (112)
Nitrate	*Thalassiosira pseudonana*	Continuous culture with 25 μM nitrate and ammonia; uptake from depletion of a 10 μM spike	83	0.75	ca. 0.15	1,491	0	Eppley and Renger (68)
^{13}N-nitrate	*Klebsiella pneumoniae*	Radioactive uptake of cells centrifugally separated through silicone oils; 45 s		4.9–4,200		1,200	1	Thayer and Huffaker (209)
Nitrate	*Monochrysis lutheri*	Technicon autoanalyzer of continuous culture effluent on 12-h dark/light cycle				1,044	0.35	Laws and Bannister (138)
Nitrate	Natural phytoplankton assemblage	Autoanalyzer analysis over 12 h after addition of 4 to 20 μM nitrate to continuous culture; kinetics based on particulate N		0.8		64.5	4	Harrison et al. (96)
Nitrate	*Isochrysis galbana*	Cadmium amalgam method for nitrate in filtrate from continuous culture, D = 0.071 h^{-1}			0.3	31	0.3	Caperon (39)
Nitrate	*Oscillatoria agardhii*	Continuous culture during continuous illumination; brucine reaction with filtrate concentrate		2–23	1.2	15.8	0.46	Zevenboom and Mur (220)
^{15}N-nitrate	Marine phytoplankton, oligotrophic	Mass spectrometry of particulate N increase with added nitrate, 6–24 h		0.04		12	0	MacIsaac and Dugdale (145)
^{15}N-nitrate	As above; eutrophic	As above		4.2		7.5	0	MacIsaac and Dugdale (145)

Continued on following page

TABLE 7—*Continued*

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells/g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity: Liters/g of cells per h	Specific affinity: At concn (μM)	
^{15}N-nitrate	Freshwater phyto-plankton	Emmission spectrometry of particulate N upon addition of nitrate in phosphate-amended light-saturated cultures over 6 h		5.2		1.5	0	Chan and Campbell (44)
Nitrate	*Aspergillus nidulans*	Loss of absorbance in perchlorate due to batch-grown mycelia		200		1.35	0	Brownlee and Arst (25)
[^{15}N]urea	*Thalassiosira pseudonana*	Mass spectrographic analysis of batch-grown cells in the presence of urea (which gave highest rate); 5 min				86	10	Horrigan and McCarthy (110)
[^{14}C]methylamine	*Pseudomonas* sp.	Radioactivity uptake by washed cells; 10 min		130		9	0	Bellion et al. (10)
[^{14}C]methylamine	*Pseudomonas* sp.	As above except with excess carbon source		12		13	0	Bellion et al. (10)
[^{14}C]methyl-ammonium	*Rhizobium* sp.	Radioactivity uptake by washed cells; 5 min				1.7	10	Gober and Kashket (82)
[^{14}C]urea	*Chlamydomonas reinhardi*	Uptake rate by washed suspensions of batch-grown cells		6.1		0.006	0	Williams and Hodson (216)
Dinitrogen	*Anabaena variabilis*	Loss of diffused N_2 according to the mass spectrum from harvested samples of batch-grown cells; 10 min		69		2.4	0	Jensen and Cox (118)
Acetylene	As above	As above using C_2H_2 according to gas chromatography		385		1.5	0	Jensen and Cox (118)

This technique has been used to measure the specific affinity of *Pseudomonas* sp. strain T2 for toluene during growth in an amino acid-limited continuous culture (A. T. Law and D. K. Button, manuscript in preparation).

Another method for obtaining the kinetics of nutrient uptake by nutrient-limited cells is to remove a sample from the reactor, mix it with a small quantity of limiting nutrient, and follow the rate of nutrient incorporation into cell material (34) according to equation 2. It was found that the nutrient-sequestering ability of the culture, *R. rubra* in this case, was significantly reduced by harvesting but that a rapid transfer procedure gave more active cells and ones that give specific affinities which agree with the values obtained from direct measurement of A_{out} (phosphate) in continuous culture.

Measurements of growth and nutrient uptake kinetics that utilize a more perfect steady state (considering cell growth, nutrient depletion, and metabolic pool size) have been based

TABLE 8. Phosphate

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells/g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity: Liters/g of cells per h	Specific affinity: At concn (μM)	
^{32}P	*Synechococcus*	Radioactivity uptake of steady-state samples, P limited, 10 to 120 s		0.3		1,060	0	Grillo and Gibson (87)
^{32}P	*Synechococcus*	Radioactivity uptake of steady-state samples, N limited, 10 to 129 s		1		12	0	Grillo and Gibson (87)
^{32}P	*Rhodotorula rubra*	Initial uptake of continuously grown cells, pH 4, 6 min		0.5–2.8	0.01	896	0.5	Button et al. (34)
^{32}P	*Rhodotorula rubra*	Initial uptake of continuously grown cells, pH 7, 6 min				280	0.5	Button et al. (34)
^{32}P	*Rhodotorula rubra*	Initial uptake of continuously grown cells, pH 7 adjusted to pH 4, 6 min				112	0.5	Button et al. (34)

Continued on following page

TABLE 8—*Continued*

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells/g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity		
						Liters/g of cells per h	At concn (μM)	
^{32}P	*Synechococcus nageli*	Radioactivity of the molybdate-reactive P in filtrate from continuous culture	62–44		0.008	788	0.006	Lang and Brown (134)
^{32}P	*Scenedesmus quadricauda*	Radioactivity of the molybdate-reactive P in filtrate from continuous culture	102–978		0.13	28.5	0.09	Lang and Bown (134)
^{32}P	*Rhodotorula rubra*	Radioactivity of the molybdate-reactive P in filtrate from continuous culture	167–900	4	0.012	480	0	Robertson and Button (189)
^{32}P	*Streptococcus faecalis*	Anaerobic radioactivity uptake, 5 min		10		434	0	Harold et al. (93)
Phosphate	*Anabaena variabilis*	With 300 μM Mg^{2+}		1.1		225	0	Healey (97)
Phosphate	*Anabaena variabilis*	With 30 μM Mg^{2+}		9.5		30	0	Healey (97)
^{32}P	*Scenedesmus quadriluca*	Radioactivity uptake from P-limited continuous culture, 45 min, pH 8.5				210	10	Peterson et al. (173)
^{32}P	*Scenedesmus quadriluca*	As above, pH 5.0				120	10	Peterson et al. (173)
^{32}P	*Candida tropicalis*	Radioactivity uptake, 10 min		4.5–1,300		132	0	Blasco et al. (15)
^{32}P	*Micrococcus lysodeikticus*	Radioactivity uptake, 2 min		4.3		100	0	Friedberg (74)
^{32}P	*Synechococcus leopoliensis*	Radioactivity uptake, 30 min		0.3		99	0.3	Rigby et al. (185)
^{32}P	*Neurospora crassa*	Radioactivity uptake, 40 s, pH 7.5		0.95		98	0	Bowman (18)
^{32}P	*Neurospora crassa*	Radioactivity uptake, 40 s, pH 5.8		3.34		64	0	Bowman (18)
^{32}P	*Rhodopseudomonas sphaeroides*	Radioactivity uptake, 10 min				75	50	Hellingwerf et al. (100)
^{32}P	*Selenastrum capricornutum*	Radioactivity of the molybdate-reactive P in continuous culture filtrate	266–833		0.018	72	0.004	Brown and Button (24)
^{32}P	*Selenastrum capricornutum*	Radioactivity uptake of batch-grown cells	55			11	3	Brown and Button (24)
Phosphate	*Anabaena flos-aquae*	^{33}P loss from medium and ^{32}P uptake by cells, 2 h	217	1.9		57	0	Nalewajko and Lean (161)
^{32}P	*Synechococcus leopoliensis*	Radioactivity uptake, 30 min, no added Ca^{2+}		2.4		5.7	0.3	Nalewajko and Lean (161)
Phosphate	*Nitzschia actinastroides*	Molybdate-reactive P in isobutanol extract from continuous culture with continuous light	1,406–2,941	1.2	0.01	34	0.4	Müller (159)
Phosphate	*Scenedesmus dimorphus*	Continuous culture, r = 0.17 h^{-1}	129			19	2.8	Kunikane et al. (132)
Phosphate	*Scenedesmus dimorphus*	Continuous culture, r = 0.04 h^{-1}	2,350			25	0.01	Kunikane et al. (132)
^{32}P	*Neurospora crassa*	Radioactivity uptake, 0–50 min		2.43 and 370		20	0	Burns and Beever (28)
^{74}As	*Streptococcus faecalis*	Anaerobic radioactivity uptake, 5 min				5.25	2	Harold and Spitz (94)
^{32}P	*Streptococcus faecalis*	Anaerobic radioactivity uptake, 5 min				4.73	1	Harold and Spitz (94)
^{32}P	*Aspergillus nidulans*	Radioactivity uptake of 50-μm germlings		9.3 and 559		4.3	0	Beever and Burns (9)
^{32}P	*Oscillatoria thiebautii*	Natural collections		0.3		0.3	0	McCarthy and Carpenter (154)

Continued on following page

TABLE 9. Minerals

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells/g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity		
						Liters/g of cells per h	At concn (μM)	
^{35}S	*Penicillium notatum*	Radioisotope uptake by washed suspensions of batch-grown cells; 60 s		3		60	0	Cuppoletti and Segel (53)
^{35}S	*Desulfobacter postgatei*	Anaerobic uptake by washed, continuously or batch-grown cells at the expense of acetate; 2 h		145		0.7	0	Ingvorsen et al. (114)
^{35}S	*Saccharomyces cerevisiae*	Uptake of radioisotope by yeast cake		42		0.37	0	Roomans et al. (191)
Silicate	*Stephanodiscus minutus*	Loss of silicate by cells grown in semicontinuous culture (silicomolybdate method)			0.12	73	0.12	Mechling and Kilham (155)
Silicate	*Stephanodiscus minutus*	As above except cells were grown in batch culture			0.88	8.1	1.0	Mechling and Kilham (155)
Silicate	*Thalassiosira weisflogii*	Rate of loss of silicate during batch growth according to chemical assay; 5 h for uptake; 25 h for growth; rates lower below 10^{-9} M Cu^{2+}	7.7	3	10	26.4	10	Rueter (193)
Silicate	*Skeletonema costatum*	Transient silicate uptake according to sodium carbonate fusion method of silicate-limited continuous culture, D = 0.036 h^{-1}; 2 h	6.8	0.7		6.8	10	Conway et al. (48)
Silicate	Seawater populations	Dissolved silicate by autoanalyzer; particulate by sodium carbonate fusion		2.9		3.9	2	Goering et al. (83)
^{45}Ca	*Saccharomyces cerevisiae*	Radioisotope uptake by yeast cake; sum of two apparent systems		710 2,500		I 15 II 3.1 Sum 18.1	0 0 0	Borst-Pauwels (17)
Magnesium	*Escherichia coli*	Batch-grown cells, magnesium deficient, atomic absorption spectroscopy	4,100		2	5.5	0	Lusk et al. (144)
^{28}Mg	*Escherichia coli*	Radioactivity uptake by batch-grown cells; 5 min		31		4.6	0	Jasper and Silver (117)
^{28}Mg	*Rhodopseudomonas capsulata*	Radioactivity uptake by batch-grown cells; 5 min		55		0.58	0	Jasper and Silver (117)
^{28}Mg	*Bacillus subtilis*	Radioactivity uptake by batch-grown cells; 5 min		250		0.31	0	Jasper and Silver (117)
^{53}Fe-schizokinen	*Anabaena* sp.	Radioisotope uptake of iron from schizokinen; 5 min		0.04		4.5	0	Lammers and Sanders-Loehr (133)
^{53}Fe-transferrin	*Neisseria meningitidis*	Radioisotope uptake of iron from transferrin;5 min		35		0.7	6	Simonson et al. (201)
Potassium	*Rhodopseudomonas capsulata*			200		2.4	0	Jasper and Silver (117)
Potassium	*Streptococcus faecalis*	Loss of intercellular K^+ in cell suspensions according to ion-specific electrode; 10 min		500		0.53	2,000	Kobayashi (125)

Continued on following page

TABLE 9—*Continued*

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells/g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity		
						Liters/g of cells per h	At concn (μM)	
$^{22}NaCl$	*Streptococcus faecalis*	Uptake of radioisotope by cell suspensions, 1 h				0.0035	2×10^4	Heefner et al. (99)
Manganese	*Rhodopseudomonas capsulata*			0.48		0.75	0	Jasper and Silver (117)
^{54}Mn	*Escherichia coli*	Radioactivity uptake by batch-grown cells, 60 min		0.2		1.2	0.2	Silver et al. (199)
Manganese	*Arthrobacter* sp.	Loss of manganese in supernatant due to absorption by agar-plate-grown cells according to atomic absorption; 4 h		125		0.056	125	Bromfield and David (22)
^{65}Zn	*Escherichia coli*	Radioactivity uptake by batch-grown cells, 10 min				2.4	10	Bucheder and Broda (26)
^{65}Zn	*Candida utilis*	Radioactivity uptake by glucose-amended batch-grown cells				0.82	1.1	Failla et al. (70)
$H_2{}^{14}CO_3$	*Scenedesmus obliquus*	Uptake of radioactivity in samples from bicarbonate-limited continuous cultures; 2 h		100		0.78	833	Azov (5)
$H_2{}^{14}CO_3$	As above	As above except without a low CO_2 adaption period				0.089	833	Jasper and Silver (117)
^{48}V	*Neurospora crassa*	Uptake of radioactivity by harvested cells; 40 s		8.2		0.54	8.3	Bowman (18)
^{63}Ni	*Methanobacterium bryantii*	Uptake of radioactivity by anaerobic suspensions; 10 min				0.11	15	Jarrell and Sprott (116)
^{63}Ni	Baker's yeast	Radioisotope uptake by yeast cake; 20 min		900		0.09	500	Fuhrmann and Rothstein (75)

on nutrient concentrations and cell population data within the reactor of continuous cultures. If all the cells are alive and the culture is steadily fed and perfectly mixed without growth on the reactor walls, then the dilution rate and the average growth rates of the organisms are truly equal (equation 17 [136]). In such systems the biomass may change in quality with growth rate, but at any particular growth rate the substrate-sequestering ability of the cells is adapted to the concentration in question. If the nutrient concentration can be accurately measured, the kinetics of both uptake and growth can be faithfully described without disturbing the system. Resulting expressions relate a range of substrate concentrations to a range of rates. The quality of biomass, although variable, is associated with each concentration-rate pair in question. These kinetics from nutrient-limited growth experiments are thought to be relevant to many dilute aquatic systems because, particularly in the case of bacteria, organism production and organism removal due to grazing appear to proceed at approximately equivalent rates (72) which results in a fairly stable steady state.

Many pragmatic considerations restrict the accuracy of steady-state limiting-nutrient concentration determinations from continuous culture (136). For example, small nutrient concentrations can be very difficult to measure in biological aquatic systems. Use of radioisotopes is complicated by the problem that residual extracellular radioactivity may be derived from, in addition to the substrate, metabolic products of it. Therefore, purification steps are required (38, 188, 189).

Dilution of isotope by background substrate, ubiquitous for most nutrients, can be accounted for when collecting growth kinetic data from continuous culture. Operation begins without added limiting nutrient to evaluate background substrate from equation 19. More conveniently, the value of the background substrate is determined from departure of the yield constant at a given growth rate at low substrate concentration where A_{out} determinations are facile but background substrates are significant; Y_{XA} should be constant with growth rate and vary with substrate according to equation 39 where A_0 = added substrate A_a + background substrate A_b (34, 189) and

$$A_{out} = A_a + \left(\frac{X_b}{Y_{XA}}\right) \frac{R}{1 - R} \quad (41)$$

Very rapid separation of cells from substrate in samples is

TABLE 10. Light

Organism	Method	$Y_{Xh\nu}$ (g of cells/ Einstein)	Kinetic constants				Reference
			K_t (μEinsteins/ $m^2 \cdot s$)	K_μ (μEinsteins/ $m^2 \cdot s$)	Specific affinity (g of $CH_2O \cdot cm^2$/g of cells · μEinstein)	Specific affinity At intensity (μEinsteins/ $m^2 \cdot s$)	
Chlorella vulgaris	$H_2{}^{14}CO_3$ uptake by batch-grown cells at a light intensity of 0.14 μEinsteins/$m^2 \cdot s$				18	1.4	Steeman-Nielsen et al. (205)
Chlorella pyrenoidosa	Oxygen evolution by batch-grown cells in flashing light ($10/10^5$ μs); 6-min incubation		208		0.19	1	Myers and Graham (160)
Scenedesmus sp.	Nutrient-sufficient cells grown in continuous culture which was adjusted in dilution rate to maintain constant population density (turbidostat)				0.041	0.7	Rhee et al. (182)
Fragilaria crotonensis	As above			12.3	0.016	12.3	Rhee et al. (182)
Anabaena variabilis	$H_2{}^{14}CO_3$ uptake of high-light-conditioned cells; 2 h		145		1.3×10^{-3}	195	Collins and Boylen (47)
	As above except 1 day after a reduction in light intensity		>600		0.23×10^{-3}	195	Collins and Boylen (47)
	As above except either 4 or 10 days after a reduction in light intensity		115		1.1×10^{-3}	195	Collins and Boylen (47)
Seawater from Baffin Bay, 10 m	$H_2{}^{14}CO_3$ uptake, 2 h			1.2	5.4×10^{-3}	1.6	Gallegos et al. (78)
Merismopedia tenuissima	Growth rate in batch culture		35[a]	22	1.38×10^{-3}	22	Konopka and Schnur (128)
Thalassiosira fluviatilis	$H_2{}^{14}CO_3$ uptake in continuous culture samples; 12-h light cycle, daytime values				4.9×10^{-4}		Laws and Bannister (138)
	As above except based on estimated light experienced by cells	16.2		30.5	7.4×10^{-4}	28	Laws and Bannister (138)
Pyrocystis noctiluca	Growth in batch culture over 4 months on 12-h light cycle			28.8	6.9×10^{-5}	28.8	Rivkin et al. (186)
Gonyaulax polyedra	Extended growth rate in batch culture		80	85	6.5×10^{-5}[b]	85	Rivkin et al. (187)
Scenedesmus quadricauda	$H_2{}^{14}CO_3$ uptake over 1 in samples from batch cultures diluted daily on a 12-h light cycle		124		2.5×10^{-5}	124	Smith (203)

[a] Assuming 11% respiration, including a 18.8-μEinstein/m^2 threshold intensity.
[b] Grown at 20 μEinsteins/m^2 per s, 56 if grown at 180.

required if the true intercellular substrate concentration is to be reflected. The half-time for such substrate loss (136) is

$$t_{1/2} = \frac{1}{D} \ln \frac{(A_0 - A_{out}) + 1/2A_{out}}{A_0 - A_{out}} \quad (42)$$

or 1.8 min for phosphate supplied at only 1.5 μM.

Organism requirements for nutrients can be supplied at a given periodicity (38). Resulting fluctuations can be treated as either an average steady state or a transient state, depending on the time frame reference. These and other questions about both the state of the biomass and the nutrient concentration that uptake rates reflect require consideration when using rate equations such as 8 and 14 and equations 24 and 34 derived from them.

Effective Concentrations

Uptake rate-controlling nutrient concentrations at the surface of organisms differ from those in bulk solution except at zero flux. An example of enhanced nutrient concentration can be found within a decaying detritus particle to the advantage of organisms that have managed to penetrate it. Exploitation of the nutrient plumes behind swimming zooplankters is another, but more controversial, example of the difference between effective nutrient concentration and

TABLE 11. Electron acceptors

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells/g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity		
						Liters/g of cells per h	At concn (μM)	
Oxygen	*Escherichia coli*	Oxygen by Clark electrode in cell suspensions harvested from oxygen-limited continuous cultures, $\mu = 0.09$	2.62	0.19		15,157	0	Rice and Hempfling (183)
Oxygen	*Escherichia coli*	As above except $\mu = 0.48$ h^{-1}			0.18 and 0.023	77,000	0	Rice and Hempfling (183)
Oxygen	*Candida utilis*	Oxygen by Teflon-covered Ag-Pb probe inserted into continuous culture, uptake rate from exhaustion rate; 15 min	1.46		1.3	3,624	0.105	Borkowski and Johnson (16)
Oxygen	*Beneckea natriegens*	Oxygen tension in gas phase over respiring cells		0.18		3,583	0	Linton et al. (143)
Oxygen	*Sphaerotilus natans*	Oxygen by Ag-Pb probe in continuous culture; growth rate computed from washout rate			0.43	1,747	0	Hao et al. (91)
Oxygen	*Sphaerotilus natans*	As above			2.2	887	0	Hao et al. (91)
Oxygen	*Torulopsis utilis*	Extrapolation of added oxygen to zero cell population in continuous culture (equation 19)	3.4–5.1			235	0	Button and Garver (36)
Oxygen	*Saccharomyces cerevisiae*	Polarographic measurements of oxygen in anaerobic cell suspensions		1		4.5	30	Terui et al. (208)
Tetrathionate	*Thiobacillus ferrooxidans*	Oxygen consumption by cell suspensions and yield in continuous culture	0.121	400		5.7	0	Eccleston and Kelly (64)
Iron	*Thiobacillus ferrooxidans*	Continuous culture, Fe^{2+} at steady state by the orthophenanthroline culture	0.002–0.005		970	16	1,050	Forshaug (M.S. thesis, University of Alaska, Fairbanks, 1983)
[^{35}S]thiosulfate	*Chlorobium vibrioforme*	Uptake of radioisotope by washed cell suspensions				0.005	100	Khanna and Nicholas (124)

that in bulk solution (140). Concentrations are larger in bulk solution than at the surface of organisms where active transport (Fig. 2) provides a continuous sink as formulated by Fick's first law. This can be written

$$A = D \frac{dA}{dt} A' \Delta t \quad (43)$$

where the amount A of substrate transported into an organism depends on the diffusivity D, the surface area of the organism A', and the time interval observed. Borkowski and Johnson (16) derived a simple relationship for spherical organisms (which seems to be limited to high-flux situations) and used it to calculate a thickness of the unstirred layer (77) of 0.6 μm as sufficient for oxygen to increase in concentration from 0.0 to 1.2 μM in a rapidly growing yeast fermentation. In agreement with this, our approximations using equations 23 and 42 and Table 6 give diffusion films of under 0.1 μm for carbohydrate-limited bacteria. These diffusion films are even thinner for nitrogen and phosphate at the surface of phytoplankton because, even though algae are larger than bacteria, both growth rates and yields are smaller. Calculations indicate that concentrations at the organism surface in all but very fast-growing systems are nearly as large as those in bulk solution, but the exact gradient depends on the mixing profile outward from the organism, which has not been verified experimentally. These gradients give a decrease in ambient substrate concentration at constant growth rate in continuous culture which we once observed (30). The observation, originally by Winne (217), that unstirred layers cause upward bias in Michaelis constants (hence downward bias in specific affinity), although relevant to the nonfluid system examined, would appear to be less dramatic for aquatic microorganisms because of larger diffusivities and turbulence. Koch and Wang (127) makes the point that, if rate-limiting transporters are buried within organisms, then diffusion to them can slow uptake rates, decrease transport efficiency, and give less curvature to the saturation relationship for nutrient uptake. Both spherical diffusion (Koch [126]) and collision frequency calculations give differences, if unimpeded by the cell enve-

TABLE 12. Vitamins

Substrate	Organism	Method and incubation time	Y_{XA} (g of cells/g of substrate)	Kinetic constants				Reference
				K_t (μM)	K_μ (μM)	Specific affinity		
						Liters/g of cells per h	At concn (μM)	
[^{14}C]thiamine	*Saccharomyces cerevisiae*	Uptake of radioisotope by centrifuged cells; 1 min		3.3×10^{-4}		1,636	3.3×10^{-1}	Theuvenet et al. (211)
Thiamine	*Cryptococcus albidus*	Extrapolation of added substrate to zero cell population in continuous culture	1.2×10^5–29.0×10^5		4.7×10^{-7}	720	1×10^{-7}	Button (30)
^{57}Co-vitamin B_{12}	*Monochrysis lutheri*	Radioactivity at steady state in continuous culture, also uptake of radioisotope; 48 h	8.5×10^9	1.9×10^{-6}	1.0×10^{-7}	1.85	0	Droop (60)

lope, of 10^2 to 10^3 in excess of the rate required for growth. Tortuous treatments of diffusive limitation abound, but none seem to define clearly the concentration at the surface of the organism and the gradients involved. The salient difficulty is in knowing the degree of mixing and hence the value of D as a function of distance outward from the surface of the organism. However, the basic point that observed Michaelis concentrations can be different from those driving reactions remains noteworthy. For example, if a lipophylic substrate partitions favorably into the membrane lipid of a hydrocarbon-oxidizing organism and bathes a membrane-bound oxidase in the resulting high-concentration microenvironment, then the apparent Michaelis constant of the enzymatic reaction would exceed K_μ for the whole cell (neglecting flux-generated gradients) by the value of the partition coefficient.

Reasonable Kinetic Constants

Combining equations 8 and 19, growth rate is given from yield and specific affinity by

$$\mu = \frac{V\,Y_{XA}}{X} = v^S\,Y_{XA} = a_A\,A_{out}\,Y_{XA} \qquad (44)$$

For a perfectly Michaelian system (32)

$$a_A{}^0 = \frac{V_{max}}{K_t} \qquad (45)$$

If transport of nutrient A is limiting growth and $\mu_{max} = V_{max}/Y_{XA}$ then

$$a_A{}^0 = \frac{\mu_{max}}{Y_{XA}\,K_t} \qquad (46)$$

At higher concentration saturation restricts the specific affinity. At the Michaelis concentration it is restricted in this hypothetical system to

$$a_A{}^{K_\mu} = \frac{a_A{}^0}{2} \qquad (47)$$

and

$$a_A{}^{K_\mu} = \frac{(\mu_{max}/2)}{K\mu\,Y_{XA}} \qquad (48)$$

where Y_{XA} is the cell yield at $\mu_{max}/2$.

For a growing bacterium to achieve a $\mu_{max} = 0.1$/h during growth on a sugar with a yield of $Y_{XA} = 1.0$ and $K_t = 0.1$ mg of A per liter, the specific affinity required is 1,000 liters/g of cells per h (500 if the Michaelis concentration K_μ is supplied and the value of $a_A{}^{Kt} = 500$ is observed). For a phosphate-limited phytoplankter with a specific affinity of 100 liters /g of cells per h and yield of 230 g of cells per g of phosphate to grow at a rate of 0.05 h^{-1}, the required phosphate concentration is 2 μg/liter, well within the range of steady-state phosphate levels observed in lakes (129).

KINETIC DATA

Organization of Tabulations for Bioelements

The ability of various organisms to collect nutrients is, in this paper, divided into classes of nutrients: organic carbon in Table 6, nitrogen in Table 7, phosphate in Table 8, minerals in Table 9, light in Table 10, electron acceptors in Table 11, and vitamins in Table 12. Data are ordered by decreasing specific affinity values. Exceptions are that comparative data from a single laboratory using common methods are sometimes grouped together. The tabulations of Harrison et al. (96), giving cellular composition under various conditions, as well as those of Bakken and Olsen (7), Dean and Rogers (56), Parsons et al. (172), and Eppley and Renger (68), were of particular use in making required conversions. Microbial mass is given in wet weight. In the absence of more detailed information, a conversion ratio of 0.30 g of dry weight/wet weight was used except for green algae, where the value was 0.25. If the specific affinity could not be calculated by using the authors' data or by making reasonable assumptions about the cell size and composition and using cell populations, the citation was not included in the tabulations of kinetic constants. Where sufficient data were available, the initial slope of the v^S/A_{out} curve was estimated to obtain the specific affinity for the substrate always referred to as A (no multiple substrate systems are listed here). For cases where only Michaelis constants for uptake and maximal velocities were reported, the approximation $a_A{}^0 = V^S{}_{max}/K_t$ was used and the observed concentration is reported as zero. Otherwise the specific affinity at the observed concentration is reported and the concentration is listed. Where available, values for the yield of cell mass from substrate consumed, as well as the Michaelis constants for growth and transport, are given.

Both methods and observation times for uptake are given

and are thought to have a profound influence on the values reported. Whereas comparative fluxes can seem to behave in a consistent way, absolute fluxes, if computed, often provide only trace quantities of substrate as first calculated from maltose uptake by *Staphylococcus aureus* (35). For example, in the absence of special precautions, the flux of phosphate into harvested cells of *R. rubra* was far below the expected value computed from growth rates, yields, and concentrations in the continuous culture from which they were harvested (136). Although the correct flux according to calculations could be obtained for this system by using special procedures, a sufficient flux of glucose was never obtained consistently which would match the computed values. Only on occasion did nutrient flux approach the expected value (188). More recently, A. T. Law, B. R. Robertson, and K. S. Craig of this laboratory (unpublished data) have routinely recorded the specific affinity of *Pseudomonas* sp. strain T2 for toluene. This organism metabolizes toluene by an inducible system so that values for the specific affinity vary from 0.01 to 500 liters/g of cells per h. Because the Michaelis constant for transport is small, the specific affinity must be large for the organism to obtain sufficient substrate for growth. Yet measurements of the specific affinity of cells which have been grown on toluene sometimes give values which indicate that such growth was, according to equation 46, impossible. Moreover, following a consistent procedure to obtain cells which are fully induced, the observed value of the specific affinity is most inconsistent. Within the literature located here, Droop (60), aside from ourselves, appears to stand alone in his attempt to compare the nutrient flux calculated from a system during growth at a nutrient-limited steady state and those measured directly from the uptake of nutrient by harvested organisms. Since values obtained by the two methods were in excellent agreement, this achievement apparently seemed to him unworthy of belaborment. This easy correspondence between flux and kinetic constants has never been our experience (see above). One cannot help but wonder, then, if the differences in the ability of the organisms to accumulate nutrient reported here rests more on the measurement than on the differences between the species. Relative differences in flux do not seem to be so problematical; hence the values for K_t are fairly consistent. However, the values for the specific affinities vary by 10^3 or more over most of the bioelements tabulated here. It is hoped that this review will help to generate focus on the absolute values of nutrient flux during studies of nutrient-limited growth kinetics.

ACKNOWLEDGMENTS

I thank members of my laboratory past and present for helpful discussions and particularly Betsy Robertson for careful experimental measurements upon which useful theoretical considerations must always be based. I thank also the many authors who took the time to answer questions about their publications and shared their recent results. Finally, I thank Carol Button for expert editorial/layout work and Nancy Amador for word processing.

Support was provided by the State of Alaska through research support to the Institute of Marine Science and by grant 808176 and others from the U.S. Environmental Protection Agency Department of Exploratory Research.

LITERATURE CITED

1. **Akpemado, K. M., and P. A. Bracquart.** 1982. Uptake of branched-chain amino acids by *Streptococcus thermophilus*. Appl. Environ. Microbiol. **45**:136–140.
2. **Amon, J. P., and R. D. Arthur.** 1980. The requirement for sodium in marine fungi: uptake and incorporation of amino acids. Bot. Mar. **23**:639–644.
3. **Anraku, Y.** 1980. Molecular organization and physiological functions of bacterial amino acid transport systems, p. 87–110. *In* R. Sato and Y. Kagawa (ed.), Transport and bioenergetics in biomembranes. Plenum Publishing Corp., New York.
4. **Azam, F., and R. E. Hodson.** 1981. Multiphasic kinetics for D-glucose uptake by assemblages of natural marine bacteria. Mar. Ecol. Prog. Ser. **6**:213–222.
5. **Azov, Y.** 1982. Effect of pH on inorganic carbon uptake in algal cultures. Appl. Environ. Microbiol. **43**:1300–1306.
6. **Azzone, G. F.** 1983. The semantics of 'chemiosmosis.' Trends Biochem. Sci. **8**:154–155.
7. **Bakken, L. R., and R. A. Olsen.** 1983. Buoyant densities and dry-matter contents of microorganisms: conversion of a measured biovolume into biomass. Appl. Environ. Microbiol. **45**:1188–1195.
8. **Bannister, T. T.** 1979. Quantitative description of steady state, nutrient-saturated algal growth, including adaptation. Limnol. Oceanogr. **24**:76–96.
9. **Beever, R. E., and D. J. W. Burns.** 1980. Phosphorus uptake, storage and utilization by fungi. Adv. Bot. Res. **8**:127–219.
10. **Bellion, E., M. E. Kent, J. C. Aud, M. Y. Alikhan, and J. A. Bolbot.** 1983. Uptake of methylamine and methanol by *Pseudomonas* sp. strain AM1. J. Bacteriol. **154**:1168–1173.
11. **Benson, S., M. Fennewald, J. Shapiro, and C. Huettner.** 1977. Fractionation of inducible alkane hydroxylose activity in *Pseudomonas putida* and characterization of hydroxylase-negative mutations. J. Bacteriol. **132**:614–621.
12. **Bienfang, P. K.** 1975. Steady state analysis of nitrate-ammonium assimilation by phytoplankton. Limnol. Oceanogr. **20**:402–411.
13. **Blanch, H. W.** 1981. Invited review. Microbial growth kinetics. Chem. Eng. Commun. **8**:181–211.
14. **Blasco, D., and H. L. Conway.** 1982. Effect of ammonium on the regulation of nitrate assimilation in natural phytoplankton populations. J. Exp. Mar. Biol. Ecol. **61**:157–168.
15. **Blasco, F., G. Ducet, and E. Azoulay.** 1976. Mise en évidence de deux systémes de transport du phosphate chez *Candida tropicalis*. Biochimie **58**:351–357.
16. **Borkowski, J. D., and M. J. Johnson.** 1967. Experimental evaluation of liquid film resistance in oxygen transport to microbial cells. Appl. Microbiol. **15**:1483–1488.
17. **Borst-Pauwels, G. W. F. H.** 1981. Ion transport in yeast. Biochim. Biophys. Acta **650**:88–127.
18. **Bowman, B. J.** 1983. Vanadate uptake in *Neurospora crassa* occurs via phosphate transport system II. J. Bacteriol. **153**:286–291.
19. **Boyaval, P., E. Moreira, and M. J. Desmazeaud.** 1983. Transport of aromatic amino acids by *Brevibacterium linens*. J. Bacteriol. **155**:1123–1129.
20. **Boylen, C. W., and J. C. Ensign.** 1970. Intracellular substrates for endogenous metabolism during long-term starvation of rod and spherical cells of *Arthrobacter crystallopoietes*. J. Bacteriol. **103**:578–587.
21. **Britten, R. J., and F. T. McClure.** 1962. The amino acid pool in *Escherichia coli*. Bacteriol. Rev. **26**:292–335.
22. **Bromfield, S. M., and D. J. David.** 1976. Sorption and oxidation of manganous ions and reduction of manganese oxide by cell suspension of a manganese oxidizing bacterium. Soil Biol. Biochem. **8**:37–43.
23. **Brooke, A. G., and M. M. Attwood.** 1984. Methylamine uptake by the facultative methylotroph *Hyphomicrobium* X. J. Gen. Microbiol. **130**:459–463.
24. **Brown, E. J., and D. K. Button.** 1979. Phosphate-limited growth kinetics of *Selenastrum capricornutum* (Chlorophyceae). J. Phycol. **15**:305–311.
25. **Brownlee, A. G., and H. N. Arst.** 1983. Nitrate uptake in *Aspergillus nidulans* and involvement of the third gene of the nitrate assimilation gene cluster. J. Bacteriol. **155**:1138–1146.
26. **Bucheder, F., and E. Broda.** 1974. Energy-dependent zinc

transport by *Escherichia coli*. Eur. J. Biochem. **45**:555–559.
27. **Bull, A. T., and C. M. Brown.** 1979. Continuous culture applications to microbial biochemistry. Int. Rev. Biochem. **21**:177–226.
28. **Burns, D. J. W., and R. E. Beever.** 1977. Kinetic characterization of the two phosphate uptake systems in the fungus *Neurospora crassa*. J. Bacteriol. **132**:511–519.
29. **Burns, D. J. W., and R. E. Beever.** 1979. Mechanisms controlling the two phosphate uptake systems in *Neurospora crassa*. J. Bacteriol. **139**:195–204.
30. **Button, D. K.** 1969. Thiamine limited steady state growth of the yeast *Cryptococcus albidus*. J. Gen. Microbiol. **58**:15–21.
31. **Button, D. K.** 1976. The influence of clay and bacteria on the concentration of dissolved hydrocarbon in saline solution. Geochim. Cosmochim. Acta **40**:435–440.
32. **Button, D. K.** 1979. On the theory of limiting nutrient control of microbial growth kinetics. Deep Sea Res. **25**:1163–1177.
33. **Button, D. K.** 1983. Differences between the kinetics of nutrient uptake by micro-organisms, growth and enzyme kinetics. Trends Biochem. Sci. **8**:121–124.
34. **Button, D. K., S. S. Dunker, and M. L. Morse.** 1973. Continuous culture of *Rhodotorula rubra*: kinetics of phosphate-arsenate uptake, inhibition, and phosphate-limited growth. J. Bacteriol. **113**:599–611.
35. **Button, D. K., J. B. Egan, W. Hengstenberg, and M. L. Morse.** 1973. Carbohydrate transport in *Staphylococcus aureus*. IV. Maltose accumulation and metabolism. Biochem. Biophys. Res. Commun. **52**:850–855.
36. **Button, D. K., and J. C. Garver.** 1966. Continuous culture of *Torulopsis utilis*: a kinetic study of oxygen limited growth. J. Gen. Microbiol. **45**:195–204.
37. **Button, D. K., and P. J. Kinney.** 1980. Unidirectional flux determinations by isotope relaxation methods: theory, p. 269–278. *In* Continuous cultivation of microorganisms, 7th symposium. Publishing House Czechoslovak Academy of Sciences, Prague.
38. **Button, D. K., B. R. Robertson, and K. S. Craig.** 1981. Dissolved hydrocarbons and related microflora in a fjordal seaport: sources, sinks, concentrations, and kinetics. Appl. Environ. Microbiol. **42**:708–719.
39. **Caperon, J.** 1968. Population growth response of *Isochrysis galbana* to nitrate variation at limiting concentrations. Ecology **49**:866–872.
40. **Caperon, J., and J. Meyer.** 1972. Nitrogen-limited growth of marine phytoplankton. II. Uptake kinetics and their role in nutrient limited growth of phytoplankton. Deep Sea Res. **19**:619–632.
41. **Carrillo, N., and R. H. Vallejos.** 1983. The light-dependent modulation of photosynthetic electron transport. Trends Biochem. Sci. **8**:52–56.
42. **Cembella, A. D., N. J. Antia, and P. J. Harrison.** 1984. The utilization of inorganic and organic phosphorous compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: part 1. Crit. Rev. Microbiol. **10**:317–391.
43. **Cembella, A. D., N. J. Antia, and P. J. Harrison.** 1984. The utilization of inorganic and organic phosphorous compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: part 2. Crit. Rev. Microbiol. **11**:13–81.
44. **Chan, Y. K., and N. E. R. Campbell.** 1978. Phytoplankton uptake and excretion of assimilated nitrate in a small Canadian Shield lake. Appl. Environ. Microbiol. **35**:1052–1060.
45. **Chassy, B. M., and J. Thompson.** 1983. Regulation of lactose-phosphoenolpyruvate-dependent phosphotransferase system and β-D-phosphogalactoside galactohydrolase activities in *Lactobacillus casei*. J. Bacteriol. **154**:1195–1203.
46. **Clarke, P. H.** 1981. Adaptation. J. Gen. Microbiol. **126**:5–20.
47. **Collins, C. D., and C. W. Boylen.** 1982. Ecological consequences of long-term exposure of *Anabaena variabilis* to shifts in environmental factors. Appl. Environ. Microbiol. **44**: 141–148.
48. **Conway, H. L., P. J. Harrison, and C. O. Davis.** 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. Mar. Biol. **35**:187–199.
49. **Cooney, C. L.** 1981. Growth of microorganisms. Biotechnology **1**:73–112.
50. **Courchesne, W. E., and B. Magasanik.** 1983. Ammonia regulation of amino acid permeases in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **3**:672–683.
51. **Cuhel, R. L., C. D. Taylor, and H. W. Jannasch.** 1981. Assimilatory sulfur metabolism in marine microorganisms: characteristics and regulation of sulfate transport in *Pseudomonas halodurans* and *Alteromonas luteoviolaceus*. J. Bacteriol. **147**:340–349.
52. **Cuppoletti, J., and I. Segel.** 1975. Kinetic analysis of membrane transport systems: equations for net velocity and isotope exchange. J. Theor. Biol. **53**:125–144.
53. **Cuppoletti, J., and I. H. Segel.** 1975. Kinetics of sulfate transport by *Penicillium notatum*. Biochemistry **14**:4712–4718.
54. **Daigger, G. T., and C. P. L. Grady, Jr.** 1982. The dynamics of microbial growth on soluble substrates. Water Res. **16**:365–382.
55. **Davis, C. O., P. J. Harrison, and R. C. Dugdale.** 1973. Continuous culture of marine diatoms under silicate limitation. I. Synchronized life cycle of *Skeletonema costatum*. J. Phycol. **9**:175–180.
56. **Dean, A. C. R., and P. L. Rogers.** 1967. The cell size and macromolecular composition of *Aerobacter aerogenes* in various systems of continuous culture. Biochim. Biophys. Acta **148**:267–279.
57. **de Vries, G. E., A. A. N. van Brussel, and A. Quispel.** 1982. Mechanism and regulation of glucose transport in *Rhizobium leguminosarum*. J. Bacteriol. **149**:872–879.
58. **Dijkuizen, L., and W. Harder.** 1979. Regulation of autotrophic and heterotrophic metabolism in *Pseudomonas oxalaticus* OX1: growth on mixtures of acetate and formate in continuous culture. Arch. Microbiol. **123**:47–53.
59. **Dills, S. S., and S. Seno.** 1983. Regulation of hexitol catabolism in *Streptococcus mutans*. J. Bacteriol. **153**:861–866.
60. **Droop, M. R.** 1968. Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. J. Mar. Biol. Assoc. U. K. **48**:689–733.
61. **Droop, M. R.** 1983. 25 years of algal growth kinetics. Bot. Mar. **26**:99–112.
62. **Droop, M. R., M. J. Mickelson, J. M. Scott, and M. F. Turner.** 1982. Light and nutrient status of algal cells. J. Mar. Biol. Assoc. U. K. **62**:403–434.
63. **Dugdale, R. C., B. H. Jones, Jr., J. J. MacIsaac, and J. J. Goering.** 1981. Adaptation of nutrient assimilation. Can. Bull. Fish. Aquat. Sci. **210**:234–250.
64. **Eccleston, M., and D. P. Kelly.** 1978. Oxidation kinetics and chemostat growth kinetics of *Thiobacillus ferro-oxidans* on tetrathionate and thiosulfate. J. Bacteriol. **134**:718–727.
65. **Ecker, D. J., and T. Emery.** 1983. Iron uptake from ferrichrome A and iron citrate in *Ustilago sphaerogena*. J. Bacteriol. **155**:616–622.
66. **Eisenberg, R. C., and P. V. Phibbs, Jr.** 1982. Characterization of an inducible mannitol-binding protein from *Pseudomonas aeruginosa*. Curr. Microbiol. **7**:229–234.
67. **Eppley, R. W., and J. L. Coatsworth.** 1968. Uptake of nitrate and nitrite by *Ditylum brightwellii*—kinetics and mechanisms. J. Phycol. **4**:151–156.
68. **Eppley, R. W., and E. H. Renger.** 1974. Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. J. Phycol. **10**:15–23.
69. **Eppley, R. W., and J. D. H. Strickland.** 1968. Kinetics of marine phytoplankton growth. Adv. Microbiol. Sea **1**:23–62.
70. **Failla, M. L., C. D. Benedict, and E. D. Weinberg.** 1976. Accumulation and storage of Zn^{2+} by *Candida utilis*. J. Gen. Microbiol. **94**:23–36.
71. **Falkowski, P. G.** 1983. Enzymology of nitrogen assimilation, p. 839–867. *In* E. J. Carpenter and D. G. Capone (ed.), Nitrogen in the marine environment. Academic Press, Inc., New York.
72. **Fenchel, T.** 1982. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. Mar. Ecol. Prog. Ser. **8**:225–231.
73. **Finan, T. M., J. M. Wood, and D. C. Jordan.** 1983. Symbiotic

properties of C_4-dicarboxylic acid transport mutants of *Rhizobium leguminosarum*. J. Bacteriol. **154**:1403–1413.
74. **Friedberg, I.** 1977. Phosphate transport in *Micrococcus lysodeikticus*. Biochim. Biophys. Acta **466**:451–460.
75. **Fuhrmann, G.-F., and A. Rothstein.** 1968. The transport of Zn^{2+}, Co^{2+} and Ni^{2+} into yeast cells. Biochim. Biophys. Acta **163**:325–330.
76. **Futai, M., and H. Kanzawa.** 1980. Biochemistry and molecular biology of proton-translocating ATPase of *Escherichia coli*, p. 57–86. *In* R. Sato and Y. Kagawa (ed.), Transport and bioenergetics in biomembranes. Plenum Publishing Corp., New York.
77. **Gains, N.** 1980. The determination of the kinetic parameters of a carrier mediated transport process in the presence of an unstirred water layer. J. Theor. Biol. **87**:559–568.
78. **Gallegos, C. L., T. Platt, W. G. Harrison, and B. Irwin.** 1983. Photosynthetic parameters of arctic marine phytoplankton: vertical variations and time scales of adaptation. Limnol. Oceanogr. **28**:698–708.
79. **Garside, C., and P. M. Glibert.** 1984. Computer modeling of ^{15}N uptake and remineralization experiments. Limnol. Oceanogr. **29**:199–204.
80. **Gaudy, A. F., Jr., A. Obayashi, and E. T. Gaudy.** 1971. Control of growth rate by initial substrate concentration at values below maximum rate. Appl. Microbiol. **22**:1041–1047.
81. **Gerloff, G. C., and F. Skoog.** 1954. Cell contents of nitrogen and phosphorus as a measure of their availability for growth of *Microcystis aeruginosa*. Ecology **35**:348–53.
82. **Gober, J. W., and E. R. Kashket.** 1983. Methylammonium uptake by *Rhizobium* sp. strain 32H1. J. Bacteriol. **153**: 1196–1201.
83. **Goering, J. J., D. M. Nelson, and J. A. Carter.** 1973. Silicic acid uptake by natural populations of marine phytoplankton. Deep Sea Res. **20**:777–789.
84. **Goldman, J. C., and P. M. Glibert.** 1982. Comparative rapid ammonium uptake by four species of marine phytoplankton. Limnol. Oceanogr. **27**:814–827.
85. **Goldman, J. C., C. D. Taylor, and P. M. Glibert.** 1981. Nonlinear time-course uptake of carbon and ammonium by marine phytoplankton. Mar. Ecol. Prog. Ser. **6**:137–148.
86. **Gregory, M. E., M. H. J. Keenan, and A. H. Rose.** 1982. Accumulation of L-asparagine by *Saccharomyces cerevisiae* X-2180. J. Gen. Microbiol. **128**:2557–2562.
87. **Grillo, J. F., and J. Gibson.** 1979. Regulation of phosphate accumulation in the unicellular cyanobacterium *Synechococcus*. J. Bacteriol. **140**:508–517.
88. **Guldberg, C. M., and P. Waage.** 1867. Etudes sur les affinites chimiques. Brogger and Christie, Christiania, Oslo.
89. **Hackstadt, T., and J. C. Williams.** 1983. pH dependence of the *Coxiella burnetii* glutamate transport system. J. Bacteriol. **154**:598–603.
90. **Hamilton, W. A., and I. R. Booth.** 1982. A consideration of the role of thermodynamic and kinetic factors in regulating lactose accumulation in *Escherichia coli*, p. 41–46. *In* A. N. Martonosi (ed.), Membranes and transport, vol. 2. Plenum Publishing Corp., New York.
91. **Hao, O. J., M. G. Richard, D. Jenkins, and H. W. Blanch.** 1983. The half-saturation coefficient for dissolved oxygen: a dynamic method for its determination and its effect on dual species competition. Biotechnol. Bioeng. **25**:403–416.
92. **Harold, F. M.** 1974. Chemiosmotic interpretation of active transport in bacteria. Ann. N.Y. Acad. Sci. **227**:297–311.
93. **Harold, F. M., R. L. Harold, and A. Abrams.** 1965. A mutant of *Streptococcus faecalis* defective in phosphate uptake, J. Biol. Chem. **240**:3145–3153.
94. **Harold, F. M., and E. Spitz.** 1975. Accumulation of arsenate, phosphate, and aspartate by *Streptococcus faecalis*. J. Bacteriol. **122**:266–277.
95. **Harrison, P. J., H. L. Conway, and R. C. Dugdale.** 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. I. Cellular chemical composition and steady-state growth kinetics of *Skeletonema costatum*. Mar. Biol. **35**:177–186.
96. **Harrison, P. J., H. L. Conway, R. W. Holmes, and C. O. Davis.** 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation. III. Cellular chemical composition and morphology of *Chaetoceros debilis*, *Skeletonema costatum*, and *Thalassiosira gravida*. Mar. Biol. **43**:19–31.
97. **Healey, F. P.** 1973. Characteristics of phosphorus deficiency in *Anabaena*. J. Phycol. **9**:383–394.
98. **Healey, F. P.** 1980. Slope of the Monod equation as an indicator of advantage in nutrient competition. Microb. Ecol. **5**:281–286.
99. **Heefner, D. L., H. Kobayashi, and F. M. Harold.** 1980. ATP-linked sodium transport in *Streptococcus faecalis*. II. Energy coupling in everted membrane vesicles. J. Biol. Chem. **255**:11403–11407.
100. **Hellingwerf, K. J., I. Friedberg, J. S. Lolkema, P. A. M. Michels, and W. N. Konigs.** 1982. Energy coupling of facilitated transport of inorganic ions in *Rhodopseudomonas sphaeroides*. J. Bacteriol. **150**:1183–1191.
101. **Henri, V.** 1903. Lois générales de l'action des diastases. Hermann, Paris.
102. **Herbert, D.** 1958. Continuous culture of microorganisms; some theoretical aspects, p. 45–52. *In* Continuous cultivation of microorganisms. A symposium. Publishing House Czechoslovak Academy of Sciences, Prague.
103. **Herbert, D., R. Elsworth, and R. C. Telling.** 1956. The continuous culture of bacteria: a theoretical and experimental study. J. Gen. Microbiol. **14**:601–622.
104. **Hodgson, D. A.** 1982. Glucose repression of carbon source uptake and metabolism in *Streptomyces coelicolor* A3 (2) and its perturbation in mutants resistant to 2-deoxyglucose. J. Gen. Microbiol. **128**:2417–2430.
105. **Höfle, M. G.** 1979. Effects of sudden temperature shifts on pure cultures of four strains of freshwater bacteria. Microb. Ecol. **5**:17–26.
106. **Höfle, M. G.** 1982. Glucose uptake of *Cytophaga johnsonae* studied in batch and chemostat culture. Arch. Microbiol. **133**:289–294.
107. **Höfle, M. G.** 1983. Long-term changes in chemostat cultures of *Cytophaga johnsonae*. Appl. Environ. Microbiol. **46**: 1045–1053.
108. **Hofstee, B. H. L.** 1952. On the evaluation of the constants V_m and K_M in enzyme reactions. Science **116**:329–331.
109. **Holm, N. P., and D. E. Armstrong.** 1981. Role of nutrient limitation and competition in controlling the populations of *Asterionella formosa* and *Microcystis aeruginosa* in semicontinuous culture. Limnol. Oceanogr. **26**:622–634.
110. **Horrigan, S. G., and J. J. McCarthy.** 1982. Phytoplankton uptake of ammonium and urea during growth of oxidized forms of nitrogen. J. Plankton Res. **4**:379–389.
111. **Hugouvieux-Cotte-Pattat, N., Y. Quesneau, and J. Robert-Baudouy.** 1983. Aldohexuronate transport system in *Erwinia carotovora*. J. Bacteriol. **154**:663–668.
112. **Hyman, M. R., and P. M. Wood** 1983. Methane oxidation by *Nitrosomonas europaea*. Biochem. J. **212**:31–37.
113. **Ingraham, J. L.** 1958. Growth of psychrophilic bacteria. J. Bacteriol. **76**:75–80.
114. **Ingvorsen, K., A. J. B. Zehnder, and B. B. Jorgensen.** 1984. Kinetics of sulfate and acetate uptake by *Desulfobacter postgatei*. Appl. Environ. Microbiol. **47**:403–408.
115. **Jannasch, H. W.** 1977. Growth kinetics of aquatic bacteria, p. 55–68. *In* F. A. Skinner and J. M. Shewan (ed.), Aquatic microbiology. Academic Press, Inc., New York.
116. **Jarrell, K. F., and G. D. Sprott.** 1982. Nickel transport in *Methanobacterium bryantii*. J. Bacteriol. **151**:1195–1203.
117. **Jasper, P., and S. Silver.** 1977. Magnesium transport in microorganisms, p. 7–47. *In* E. D. Weinberg (ed.), Microorganisms and minerals. Marcel Dekker, New York.
118. **Jensen, B. B., and R. P. Cox.** 1983. Direct measurements of steady-state kinetics of cyanobacterial N_2 uptake by membrane-leak mass spectrometry and comparisons between nitrogen fixation and acetylene reduction. Appl. Environ. Microbiol. **45**:1331–1337.
119. **Johnson, M. J.** 1967. Aerobic microbial growth at low oxygen concentrations. J. Bacteriol. **94**:101–108.

120. **Jones, K. L., and M. E. Rhodes-Roberts.** 1981. The survival of marine bacteria under starvation conditions. J. Appl. Bacteriol. **50**:247–258.
121. **Käppeli, O., and W. R. Finnerty.** 1979. Partition of alkane by an extracellular vesicle derived from *Acinetobacter*. J. Bacteriol. **140**:707–712.
122. **Keenan, M. H. J., A. H. Rose, and B. W. Silverman.** 1982. Effect of plasma-membrane phospholipid unsaturation on solute transport into *Saccharomyces cerevisiae* NCYC 366. J. Gen. Microbiol. **128**:2547–2556.
123. **Kemp, C. W., S. A. Robrish, M. A. Curtis, S. A. Sharer, and W. H. Bowen.** 1982. Application of a competition model to the growth of *Streptococcus mutans* and *Streptococcus sanguis* in binary continuous culture. Appl. Environ. Microbiol. **45**:1277–1282.
124. **Khanna, S., and D. J. D. Nicholas.** 1982. Utilization of tetrathionate and ^{35}S-labelled thiosulphate by washed cells of *Chlorobium vibrioforme* f. sp. *thiosulfatophilum*. J. Gen. Microbiol. **128**:1027–1034.
125. **Kobayashi, H.** 1982. Second system for potassium transport in *Streptococcus faecalis*. J. Bacteriol. **150**:506–511.
126. **Koch, A. L.** 1971. The adaptive responses of *Escherichia coli* to a feast and famine existence. Adv. Microb. Physiol. **6**:147–217.
127. **Koch, A. L., and C. H. Wang.** 1982. How close to the theoretical diffusion limit do bacterial uptake systems function? Arch. Microbiol. **131**:36–42.
128. **Konopka, A., and M. Schnur.** 1980. Effect of light intensity on macromolecular synthesis in cyanobacteria. Microb. Ecol. **6**:291–301.
129. **Koschel, R.** 1980. Untersuchungen zur Phosphataffinität des Planktons in der euphotischen Zone von Seen. Limnologica **12**:141–145.
130. **Kotyk, A.** 1982. Sugar transport in yeast. Membr. Transp. **2**:55–59.
131. **Kundig, W., D. F. Kundig, B. Anderson, and S. Roseman.** 1966. Restoration of active transport of glyconides in *Escherichia coli* by a component of a phosphotransfer system. J. Biol. Chem. **241**:32–43.
132. **Kunikane, S., M. Kaneko, and R. Maehara.** 1981. Steady state analysis of algal cultures grown under simultaneous limitation of nitrogen and phosphorus. Verh. Int. Verein. Limnol. **21**:1454–1457.
133. **Lammers, P. J., and J. Sanders-Loehr.** 1982. Active transport of ferric schizokinen in *Anabaena* sp. J. Bacteriol. **151**:288–294.
134. **Lang, D. S., and E. J. Brown.** 1981. Phosphorus-limited growth of a green alga and a blue-green alga. Appl. Environ. Microbiol. **42**:1002–1009.
135. **Law, A. T., and D. K. Button.** 1977. Multiple-carbon-source-limited growth kinetics of a marine coryneform bacterium. J. Bacteriol. **129**:115–123.
136. **Law, A. T., B. R. Robertson, S. S. Dunker, and D. K. Button.** 1976. On describing microbial growth kinetics from continuous culture data: some general considerations, observations, and concepts. Microb. Ecol. **2**:261–283.
137. **Lawry, N. H., and R. D. Simon.** 1982. The normal and induced occurrence of cyanophycin inclusion bodies in several blue-green algae. J. Phycol. **18**:391–399.
138. **Laws, E. A., and T. T. Bannister.** 1980. Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phyto-plankton growth in the ocean. Limnol. Oceanogr. **25**:457–473.
139. **Laws, E., and J. Caperon.** 1976. Carbon and nitrogen metabolism by *Monochrysis lutheri*: measurement of growth-rate dependent respiration rates. Mar. Biol. **36**:85–97.
140. **Lehman, J. T., and D. Scavia.** 1982. Microscale nutrient patches produced by zooplankton. Proc. Natl. Acad. Sci. U.S.A. **79**:5001–5005.
141. **Leo, A., C. Hansch, and D. Elkins.** 1971. Partition coefficients and their uses. Chem. Rev. **71**:525–616.
142. **Licko, V.** 1972. Some biochemical threshold mechanisms. Bull. Math. Biophys. **34**:103–112.
143. **Linton, J. D., A. T. Bull, and D. E. F. Harrison.** 1977. Determination of the apparent K_m for oxygen of *Beneckea natriegens* using the respirograph technique. Arch. Microbiol. **114**:111–113.
144. **Lusk, J. E., R. J. P. Williams, and E. P. Kennedy.** 1968. Magnesium and the growth of *Escherichia coli*. J. Biol. Chem. **243**:2618–2624.
145. **MacIsaac, J. J., and R. C. Dugdale.** 1969. The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. Deep Sea Res. **16**:45–57.
146. **MacIsaac, J. J., and R. C. Dugdale.** 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep Sea Res. **19**:209–232.
147. **Mackereth, F. J.** 1953. Phosphorus utilization by *Asterionella formosa* Hass. J. Exp. Bot. **4**:296–313.
148. **Maestrini, S. Y., and D. J. Bonin.** 1981. Competition among phytoplankton based on inorganic macronutrients. Can. Bull. Fish. Aquat. Sci. **210**:264–278.
149. **Mandelstam, J., and H. Halvorson.** 1960. Turnover of protein and nucleic acid in soluble and ribosome fractions of nongrowing *Escherichia coli*. Biochim. Biophys. Acta **40**:43–49.
150. **Marr, A. G., E. H. Nilson, and D. J. Clark.** 1963. Maintenance requirement of *Escherichia coli*. Ann. N.Y. Acad. Sci. **102**:536–548.
151. **Martonosi, A. N. (ed.).** 1982. Membranes and transport, vol. 1 and 2. Plenum Publishing Corp., New York.
152. **Matin, A., and H. Veldkamp.** 1978. Physiological basis of the selective advantage of a *Spirillum* sp. in a carbon-limited environment. J. Gen. Microbiol. **105**:187–197.
153. **McAllister, C. F., and J. E. Lepo.** 1982. Succinate transport by free-living forms of *Rhizobium japonicum*. J. Bacteriol. **153**:1155–1162.
154. **McCarthy, J. J., and E. J. Carpenter.** 1979. *Oscillatoria (Trichodesmium) thiebautii* (Cyanophyta) in the central North Atlantic Ocean. J. Phycol. **15**:75–82.
155. **Mechling, J. A., and S. S. Kilham.** 1982. Temperature effects on silicon limited growth of the Lake Michigan diatom *Stephanodiscus minutus* (Bacillariophyceae). J. Phycol. **18**:199–205.
156. **Michaelis, L., and M. M. L. Menten.** 1913. Die Kinetik der Invertinwirkung. Biochem. Z. **49**:333–369.
157. **Monod, J.** 1942. Recherches sur la croissance des cultures bactériennes. Hermann, Paris.
158. **Monod, J.** 1950. La technique de culture continue. Théory et applications. Ann. Inst. Pasteur Paris **79**:390–410.
159. **Müller, H.** 1972. Growth and phosphate requirements of *Nitzschia actinastroides* (Lemm.) v. Goor in batch and chemostat culture under phosphate limitation. Arch. Hydrobiol. **4**(Suppl. 30):399–434.
160. **Myers, J., and J.-R. Graham.** 1971. The photosynthetic unit in *Chlorella* measured by repetitive short flashes. Plant Physiol. **48**:282–286.
161. **Nalewajko, C., and D. R. S. Lean.** 1978. Phosphorus kinetics—algal growth relationships in batch cultures. Mitt. Int. Ver. Theor. Angew. Limnol. **21**:184–192.
162. **Neilands, J. B.** 1981. Microbial iron compounds. Annu. Rev. Biochem. **50**:715–731.
163. **Nelson, H., and R. D. Simon.** 1982. The normal and induced occurrence of cyanophycin inclusion bodies in several blue-green algae. J. Phycol. **18**:391–399.
164. **Newsholme, E. A., and B. Crabtree.** 1981. Flux-generating and regulatory steps in metabolic control. Trends Biochem. Sci. **6**:53–56.
165. **Novick, A.** 1955. Growth of bacteria. Annu. Rev. Microbiol. **9**:97–109.
166. **Nugent, H. A.** 1984. Photosynthetic electron transport in plants and bacteria. Trends Biochem. Sci. **9**:354–357.
167. **O'Neill, J. G., and J. F. Wilkinson.** 1977. Oxidation of ammonia by methane-oxidizing bacteria and the effects of ammonia on methane oxidation. J. Gen. Microbiol. **100**:407–412.
168. **Paasche, E.** 1973. Silicon and the ecology of marine plankton diatoms. II. Silicate-uptake kinetics in five diatom species. Mar. Biol. **19**:262–269.

169. **Paerl, H. W.** 1984. Cyanobacterial carotenoids: their roles in maintaining optimal photosynthetic production among aquatic bloom forming genera. Oecologia **61**:143–149.
170. **Paris, D. F., W. C. Steen, G. L. Baughman, and J. T. Barnett, Jr.** 1981. Second-order model to predict microbial degradation of organic compounds in natural waters. Appl. Environ. Microbiol. **41**:603–609.
171. **Parsons, T. R., and M. Takahashi.** 1973. Biological oceanographic processes. Pergamon Press, Oxford.
172. **Parsons, T. R., M. Takahashi, and B. Hargrave.** 1977. Biological oceanographic processes. Pergamon Press, New York.
173. **Peterson, H. G., F. P. Healey, and R. Wagemann.** 1984. Metal toxicity to algae: a highly pH dependent phenomenon. Can. J. Fish. Aquat. Sci. **41**:974–979.
174. **Pirt, S. J.** 1965. The maintenance energy of bacteria in growing cultures. Proc. R. Soc. London Ser. B **163**:224–231.
175. **Pirt, S. J.** 1975. Principles of microbe and cell cultivation. Blackwell, Oxford.
176. **Pirt, S. J.** 1982. Maintenance energy: a general model for energy-limited and energy-sufficient growth. Arch. Microbiol. **133**:300–302.
177. **Platt, T., and A. D. Jassby.** 1976. The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton. J. Phycol. **12**:421–430.
178. **Plowman, K. M.** 1972. Enzyme kinetics. McGraw-Hill, New York.
179. **Raven, J. A.** 1980. Nutrient transport in microalgae. Adv. Microb. Physiol. **21**:47–226.
180. **Ray, W. J., Jr.** 1983. Rate-limiting step: a quantitative definition. Application to steady-state enzymic reactions. Biochemistry **22**:4625–4637.
181. **Rhee, G. Y.** 1981. The effect of environmental factors on phytoplankton growth: light and the interactions of light with nitrate limitation. Limnol. Oceanogr. **26**:649–659.
182. **Rhee, G. Y., I. J. Gotham, and S. W. Chisholm.** 1981. Use of cyclostat cultures to study phytoplankton ecology. Contin. Cult. Cells **2**:159–186.
183. **Rice, C. W., and W. P. Hempfling.** 1978. Oxygen-limited continuous culture and respiratory energy conservation in *Escherichia coli*. J. Bacteriol. **134**:115–124.
184. **Rickenberg, H. V., G. N. Cohen, J. Buttin, and J. Monod.** 1956. La galactoside-permease d'*Escherichia coli*. Ann. Inst. Pasteur Paris **91**:829–857.
185. **Rigby, C. H., S. R. Craig, and K. Budd.** 1980. Phosphate uptake by *Synechococcus leopoliensis* (Cyanophyceae): enhancement by calcium ion. J. Phycol. **16**:389–393.
186. **Rivkin, R. B., H. H. Seliger, E. Swift, and W. H. Biggley.** 1982. Light-shade adaptation by the oceanic dinoflagellates *Pyrocystis noctiluca* and *P. fusiformis*. Mar. Biol. **68**:181–191.
187. **Rivkin, R. B., M. A. Voytek, and H. H. Seliger.** 1982. Phytoplankton division rates in light-limited environments: two adaptations. Science **215**:1123–1125.
188. **Robertson, B. R., and D. K. Button.** 1977. Low substrate concentration determinations for nutrient limited growth kinetic studies. II. ^{14}C-glucose analysis by glucose oxidase thin layer chromatography. Mar. Sci. Commun. **3**:35–43.
189. **Robertson, B. R., and D. K. Button.** 1979. Phosphate-limited continuous culture of *Rhodotorula rubra*: kinetics of transport, leakage, and growth. J. Bacteriol. **138**:884–895.
190. **Rogers, P. J., and P. R. Stewart.** 1974. Energetic efficiency and maintenance energy characteristics of *Saccharomyces cerevisiae* (wild type and petite) and *Candida parapsilosis* grown aerobically and microaerobically in continuous culture. Arch. Microbiol. **99**:25–46.
191. **Roomans, G. M., G. A. J. Kuypers, A. P. R. Theuvenet, and G. W. F. H. Borst-Pauwels.** 1979. Kinetics of sulfate uptake by yeast. Biochim. Biophys. Acta **551**:197–206.
192. **Rosen, B. P.** 1978. Bacterial transport. Marcel Dekker, New York.
193. **Rueter, J. G., Jr.** 1983. Effect of copper on growth, silicic acid uptake and soluble pools of silicic acid in the marine diatom, *Thalassiosira weisflogii* (Bacillariophyceae). J. Phycol. **19**:101–104.
194. **Russell, L. M., and H. Rosenberg.** 1979. Linked transport of phosphate, potassium ions and protons in *Escherichia coli*. Biochem. J. **184**:13–21.
195. **Schlanderer, G., W. Held, J. Reimann, and H. Dellweg.** 1978. Metabolic changes during substrate shifts in continuous cultures of the yeast *Candida boidinii* variant 60. J. Appl. Microbiol. Biotechnol. **6**:133–144.
196. **Schweizer, H., M. Argast, and W. Boos.** 1982. Characteristics of a binding protein-dependent transport system for *sn*-glycerol-3-phosphate in *Escherichia coli* that is part of the *pho* regulon. J. Bacteriol. **150**:1154–1163.
197. **Segal, H. L.** 1959. The development of enzyme kinetics, p. 1–48. *In* P. D. Boyer, H. Lardy, and K. Myrbäk (ed.), The enzymes, vol. 1. Academic Press, Inc., New York.
198. **Shehata, T. E., and A. G. Marr.** 1971. Effect of nutrient concentration on the growth of *Escherichia coli*. J. Bacteriol. **107**:210–216.
199. **Silver, S., P. Johnseine, and K. King.** 1970. Manganese active transport in *Escherichia coli*. J. Bacteriol. **104**:1299–1306.
200. **Simkins, S., and M. Alexander.** 1984. Models for mineralization kinetics with the variables of substrate concentration and population density. Appl. Environ. Microbiol. **47**:1299–1306.
201. **Simonson, C., D. Brener, and I. W. DeVoe.** 1982. Expression of a high-affinity mechanism for acquisition of transferrin iron by *Neisseria meningitidis*. Infect. Immun. **36**:107–113.
202. **Slee, A. M., and J. M. Tanzer.** 1982. Sucrose transport by *Streptococcus mutans*. Evidence for multiple transport systems. Biochim. Biophys. Acta **692**:415–424.
203. **Smith, V. H.** 1983. Light and nutrient dependence of photosynthesis by algae. J. Phycol. **19**:306–313.
204. **Southhamer, A. H.** 1979. The search for correlation between theoretical and experimental growth yields. Int. Rev. Biochem. **21**:1–47.
205. **Steeman-Nielsen, E., V. K. Hansen, and E. G. Jørgensen.** 1962. The adaptation to different light intensities in *Chlorella vulgaris* and the time dependence on transfer to a new light intensity. Physiol. Plant. **15**:505–517.
206. **Sullivan, C. W.** 1979. Diatom mineralization of silicic acid. IV. Kinetics of soluble Si pool formation in exponentially growing and synchronized *Navicula pelliculosa*. J. Phycol. **15**:210–216.
207. **Talling, J. F.** 1957. Photosynthetic characteristics of some freshwater diatoms in relation to underwater radiation. New Phytol. **56**:29–50.
208. **Terui, G., N. Konno, and M. Sase.** 1960. Analysis of the behaviors of some industrial microbes toward oxygen. 1. Effect of oxygen concentration upon the rates of oxygen adaptation and metabolism of yeasts. Technol. Rep. Osaka Univ. **10**:527–544.
209. **Thayer, J. R., and R. C. Huffaker.** 1981. Use of ^{13}N-nitrate to study nitrate transport in *Klebsiella pneumoniae*. Adv. Chem. Ser. **197**:341–349.
210. **Thayer, J. R., and M. L. Wheelis.** 1982. Active transport of benzoate in *Pseudomonas putida*. J. Gen. Microbiol. **128**:1749–1753.
211. **Theuvenet, A. P. R., W. M. H. Van De Wijngaard, and G. W. F. H. Borst-Pauwels.** 1983. 9-Aminoacridine, a fluorescent probe of the thiamine carrier in yeast cells. Biochim. Biophys. Acta **730**:255–262.
212. **Tilman, D., and S. S. Kilham.** 1976. Phosphate and silicate growth and uptake kinetics of the diatoms *Asterionella formosa* and *Cyclotella meneghiniana* in batch and semicontinuous culture. J. Phycol. **12**:375–383.
213. **Underhill, P. A.** 1977. Nitrate uptake and colonial variability in the neritic diatom *Biddulphia aurita*. J. Phycol. **13**:170–176.
214. **van der Kooij, D., A. Visser, and W. A. M. Hijnen.** 1980. Growth of *Aeromonas hydrophila* at low concentrations of substrates added to tap water. Appl. Environ. Microbiol. **39**:1198–1204.
215. **Van Es, F. B., and L.-A. Meyer-Reil.** 1982. Biomass and metabolic activity of heterotrophic marine bacteria. Adv. Microb. Ecol. **6**:111–170.
216. **Williams, S. K., II, and R. C. Hodson.** 1977. Transport of urea at low concentrations in *Chlamydomonas reinhardi*. J. Bacte-

riol. **130**:266–273.
217. **Winne, D.** 1973. Unstirred layer source of biased Michaelis constants in membrane transport. Biochim. Biophys. Acta **298**:27–31.
218. **Wood, A. P., and D. P. Kelly.** 1982. Kinetics of sugar transport in *Thiobacillus* A2. Arch. Microbiol. **131**:156–159.
219. **Zebrower, M., and P. A. Loach.** 1982. Efficiency of light-driven metabolite transport in the photosynthetic bacterium *Rhodospirillum rubrum*. J. Bacteriol. **150**:1322–1328.
220. **Zevenboom, W., and L. R. Mur.** 1979. Influence of growth rate on short term and steady state nitrate uptake by nitrate-limited *Oscillatoria agardhii*. FEMS Microbiol. Lett. **6**:209–212.